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09/670756

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Sir:

Transmitted herewith for filing is the continuation-in-part patent application of

Inventor(s): Kenneth Rhodes, Maria Betty, Huai-Ping Ling, and Wenqian An

For: POTASSIUM CHANNEL INTERACTORS AND USES THEREFOR

Enclosed are:

☒ This is a request for filing a ☒ continuation-in-part ☐ divisional application under 37 CFR 1.53(b), of pending prior application serial no. 09/399,913, filed on September 21, 1999 entitled POTASSIUM CHANNEL INTERACTORS AND USES THEREFOR.

☒ 148 pages of specification, 14 pages of claims, 1 page of abstract.

☒ 48 sheets of formal drawings (Figures 1-43).

☒ An unexecuted Declaration, Petition and Power of Attorney.

☒ 92 pages of sequence listing (numbered 1-92).

☒ Transmittal Letter for Diskette of Sequence Listing.

☒ Diskette Containing Sequence Listing.

☒ Statement of Limited Recognition Under 37 C.F.R. §10.9(b)

The filing fee has been calculated as shown below:

	(Col. 1)	(Col. 2)
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TOTAL CLAIMS	54- 20	=34
INDEP. CLAIMS	2 - 3	=0
<input checked="" type="checkbox"/> MULTIPLE DEPENDENT CLAIMS PRESENTED		

* If the difference in Col. 2 is less than zero, enter "0" in Col. 2.

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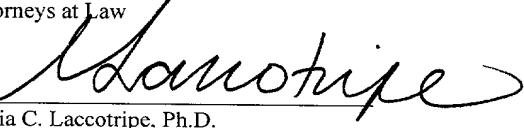
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- ☐ Any additional filing fees required under 37 C.F.R. 1.16.
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- ☒ Address all future communications (May only be completed by applicant, or attorney or agent of record) to Amy E. Mandragouras at **Customer Number: 000959** whose address is:

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POTASSIUM CHANNEL INTERACTORS AND USES THEREFOR

Related Applications

This application claims priority to U.S. provisional Application No. 60/110,033,
5 filed on November 25, 1998, U.S. provisional Application No. 60/109,333, filed on
November 20, 1998, U.S. provisional Application No. 60/110,277, filed on November
30, 1998, U.S. Patent Application No.: 09/298,731, filed on April 23, 1999, U.S. Patent
Application No.: 09/350,614, filed on July 9, 1999, U.S. Patent Application No.:
09/350,874, filed on July 9, 1999, U.S. Patent Application No.: 09/399,913, filed on
10 September 21, 1999, U.S. Patent Application No.: 09/400,492, filed on September 21,
1999, and PCT Application No. PCT/US99/27428, filed on November 19, 1999,
incorporated herein in their entirety by this reference.

Background of the Invention

15 Mammalian cell membranes are important to the structural integrity and activity
of many cells and tissues. Of particular interest in membrane physiology is the study of
trans-membrane ion channels which act to directly control a variety of pharmacological,
physiological, and cellular processes. Numerous ion channels have been identified
including calcium, sodium, and potassium channels, each of which have been
20 investigated to determine their roles in vertebrate and insect cells.

Because of their involvement in maintaining normal cellular homeostasis, much
attention has been given to potassium channels. A number of these potassium channels
open in response to changes in the cell membrane potential. Many voltage-gated
potassium channels have been identified and characterized by their electrophysiological
25 and pharmacological properties. Potassium currents are more diverse than sodium or
calcium currents and are further involved in determining the response of a cell to
external stimuli. The diversity of potassium channels and their important physiological
role highlights their potential as targets for developing therapeutic agents for various
diseases.

30 One of the best characterized classes of potassium channels are the voltage-gated
potassium channels. The prototypical member of this class is the protein encoded by the
Shaker gene in *Drosophila melanogaster*. Proteins of the Shal or Kv4 family are a type
of voltage-gated potassium channels that underlies many of the native A type currents
that have been recorded from different primary cells. Kv4 channels have a major role in

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the repolarization of cardiac action potentials. In neurons, Kv4 channels and the A currents they may comprise play an important role in modulation of firing rate, action potential initiation and in controlling dendritic responses to synaptic inputs.

The fundamental function of a neuron is to receive, conduct, and transmit
 5 signals. Despite the varied purpose of the signals carried by different classes of neurons, the form of the signal is always the same and consists of changes in the electrical potential across the plasma membrane of the neuron. The plasma membrane of a neuron contains voltage-gated cation channels, which are responsible for propagating this electrical potential (also referred to as an action potential or nerve impulse) across and
 10 along the plasma membrane.

The Kv family of channels includes, among others: (1) the delayed-rectifier potassium channels, which repolarize the membrane after each action potential to prepare the cell to fire again; and (2) the rapidly inactivating (A-type) potassium channels, which are active predominantly at subthreshold voltages and and act to reduce
 15 the rate at which excitable cells reach firing threshold. In addition to being critical for action potential conduction, Kv channels also control the response to depolarizing, *e.g.*, synaptic, inputs and play a role in neurotransmitter release. As a result of these activities, voltage-gated potassium channels are key regulators of neuronal excitability (Hille B., *Ionic Channels of Excitable Membranes*, Second Edition, Sunderland, MA:
 20 Sinauer, (1992)).

There is tremendous structural and functional diversity within the Kv potassium channel superfamily. This diversity is generated both by the existence of multiple genes and by alternative splicing of RNA transcripts produced from the same gene. Nonetheless, the amino acid sequences of the known Kv potassium channels show high
 25 similarity. All appear to be comprised of four, pore forming α -subunits and some are known to have four cytoplasmic (β -subunit) polypeptides (Jan L.Y. *et al.* (1990) *Trends Neurosci* 13:415-419, and Pongs, O. *et al.* (1995) *Sem Neurosci.* 7:137-146). The known Kv channel (α -subunits fall into four sub-families named for their homology to channels first isolated from *Drosophila*: the Kv1, or *Shaker*-related subfamily; the Kv2,

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or *Shab*-related subfamily; the Kv3, or *Shaw*-related subfamily; and the Kv4, or *Shal*-related subfamily.

Kv4.2 and Kv4.3 are examples of Kv channel (α -subunits of the *Shal*-related subfamily. Kv4.3 has a unique neuroanatomical distribution in that its mRNA is highly
5 expressed in brainstem monoaminergic and forebrain cholinergic neurons, where it is involved in the release of the neurotransmitters dopamine, norepinephrine, serotonin, and acetylcholine.

This channel is also highly expressed in cortical pyramidal cells and in interneurons. (Serdio P. *et al.* (1996) *J. Neurophys* 75:2174-2179). Interestingly, the
10 Kv4.3 polypeptide is highly expressed in neurons which express the corresponding mRNA. The Kv4.3 polypeptide is expressed in the somatodendritic membranes of these cells, where it is thought to contribute to the rapidly inactivating K⁺ conductance. Kv4.2 mRNA is widely expressed in brain, and the corresponding polypeptide also appears to be concentrated in somatodendritic membranes where it also contributes to
15 the rapidly inactivating K⁺ conductance (Sheng *et al.* (1992) *Neuron* 9:271-84). These somatodendritic A-type Kv channels, like Kv4.2 and Kv4.3, are likely involved in processes which underlie learning and memory, such as integration of sub-threshold synaptic responses and the conductance of back-propagating action potentials (Hoffman D.A. *et al.* (1997) *Nature* 387:869-875).

20 Thus, proteins which interact with and modulate the activity of potassium channel proteins *e.g.*, potassium channels having a Kv4.2 or Kv4.3 subunit, provide novel molecular targets to modulate neuronal or cardiac excitability, *e.g.*, action potential conduction, somatodendritic excitability and neurotransmitter release, in cells expressing these channels. In addition, detection of genetic lesions in the gene encoding
25 these proteins could be used to diagnose and treat central nervous system disorders such as epilepsy, spinocerebellar ataxia, anxiety, depression, age-related memory loss, migraine, obesity, Parkinsons disease or Alzheimer's disease; or cardiovascular disorders such as heart failure, hypertension, atrial fibrillation, dilated cardiomyopathy, idiopathic cardiomyopathy, or angina.

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Summary of the Invention

The present invention is based, at least in part, on the discovery of novel nucleic acid molecules which encode gene products that interact with potassium channel proteins or possess substantial homology to the gene products of the invention that

5 interact with potassium channel proteins (paralogs). Potassium channel proteins are, for example, potassium channels having a Kv4.2 or Kv4.3 subunit. The nucleic acid molecules of the invention and their gene products are referred to herein as "Potassium Channel Interacting Proteins", "PCIP", or "KChIP" nucleic acid and protein molecules. The PCIP proteins of the present invention interact with, *e.g.*, bind to a potassium

10 channel protein, modulate the activity of a potassium channel protein, and/or modulate a potassium channel mediated activity in a cell, *e.g.*, a neuronal or cardiac cell. The PCIP molecules of the present invention are useful as modulating agents to regulate a variety of cellular processes, *e.g.*, neuronal or cardiac cell processes. Accordingly, in one aspect, this invention provides isolated nucleic acid molecules encoding PCIP proteins

15 or biologically active portions thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection of PCIP-encoding nucleic acids.

In one embodiment, a PCIP nucleic acid molecule of the invention is at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more identical to the nucleotide sequence (*e.g.*, to the entire length of the nucleotide sequence) shown in SEQ

20 ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID

25 NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, or a complement thereof.

In another preferred embodiment, the isolated nucleic acid molecule includes the

30 nucleotide sequence shown SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ

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ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or a complement thereof. In another preferred embodiment, the nucleic acid molecule includes a fragment of at least 300, 350, 400, 426, 471, or 583 nucleotides of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or a complement thereof.

In another embodiment, a PCIP nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO: 2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994. In a preferred embodiment, a PCIP nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more identical to the amino acid sequence of SEQ ID NO: 2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID

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NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or
 5 98994.

In another preferred embodiment, an isolated nucleic acid molecule encodes the amino acid sequence of 1v, 9q, p19, W28559, KChIP4a, KChIP4b, 33b07, 1p, and rat 7s protein. In yet another preferred embodiment, the nucleic acid molecule includes a nucleotide sequence encoding a protein having the amino acid sequence of SEQ ID NO:
 10 2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID
 15 NO:70, or SEQ ID NO:72, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994. In yet another preferred embodiment, the nucleic acid molecule is at least 426, 471, or 583 nucleotides in length and encodes a protein having
 20 a PCIP activity (as described herein).

Another embodiment of the invention features nucleic acid molecules, preferably PCIP nucleic acid molecules, which specifically detect PCIP nucleic acid molecules relative to nucleic acid molecules encoding non-PCIP proteins. For example, in one embodiment, such a nucleic acid molecule is at least 426, 400-450, 471, 450-500, 500-
 25 550, 583, 550-600, 600-650, 650-700, 700-750, 750-800 or more nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ
 30 ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID

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NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, or
 5 a complement thereof. In preferred embodiments, the nucleic acid molecules are at least 15 (*e.g.*, contiguous) nucleotides in length and hybridize under stringent conditions to nucleotides 93-126, 360-462, 732-825, 1028-1054, or 1517-1534 of SEQ ID NO:7. In other preferred embodiments, the nucleic acid molecules comprise nucleotides 93-126, 360-462, 732-825, 1028-1054, or 1517-1534 of SEQ ID NO:7.

10 In other preferred embodiments, the nucleic acid molecules are at least 15 (*e.g.*, contiguous) nucleotides in length and hybridize under stringent conditions to nucleotides 1-14, 49-116, 137-311, 345-410, 430-482, 503-518, 662-693, 1406-1421, 1441-1457, 1478-1494, or 1882-1959 of SEQ ID NO:13. In other preferred embodiments, the nucleic acid molecules comprise nucleotides 1-14, 49-116, 137-311, 345-410, 430-482,
 15 503-518, 662-693, 1406-1421, 1441-1457, 1478-1494, or 1882-1959 of SEQ ID NO:13.

In preferred embodiments, the nucleic acid molecules are at least 15 (*e.g.*, contiguous) nucleotides in length and hybridize under stringent conditions to nucleotides 932-1527, 1548-1765, 1786-1871, 1908-2091, 2259-2265, or 2630-2654 of SEQ ID NO:35. In other preferred embodiments, the nucleic acid molecules comprise
 20 nucleotides 932-1527, 1548-1765, 1786-1871, 1908-2091, 2259-2265, or 2630-2654 of SEQ ID NO:35.

In other preferred embodiments, the nucleic acid molecule encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12,
 25 SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72 or an amino acid sequence encoded by the DNA insert of the
 30 plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950,

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98951, 98991, 98993, or 98994, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71 under stringent conditions.

Another embodiment of the invention provides an isolated nucleic acid molecule which is antisense to a PCIP nucleic acid molecule, *e.g.*, the coding strand of a PCIP nucleic acid molecule.

Another aspect of the invention provides a vector comprising a PCIP nucleic acid molecule. In certain embodiments, the vector is a recombinant expression vector. In another embodiment, the invention provides a host cell containing a vector of the invention. The invention also provides a method for producing a protein, preferably a PCIP protein, by culturing in a suitable medium, a host cell, *e.g.*, a mammalian host cell such as a non-human mammalian cell, of the invention containing a recombinant expression vector, such that the protein is produced.

Another aspect of this invention features isolated or recombinant PCIP proteins and polypeptides. In one embodiment, the isolated protein, preferably a PCIP protein, includes at least one calcium binding domain. In a preferred embodiment, the protein, preferably a PCIP protein, includes at least one calcium binding domain and has an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more identical to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942,

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SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72.

In another embodiment, the invention features an isolated protein, preferably a PCIP protein, which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more identical to a nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or a complement thereof.

The proteins of the present invention or biologically active portions thereof, can be operatively linked to a non-PCIP polypeptide (*e.g.*, heterologous amino acid sequences) to form fusion proteins. The invention further features antibodies, such as monoclonal or polyclonal antibodies, that specifically bind proteins of the invention, preferably PCIP proteins. In addition, the PCIP proteins or biologically active portions thereof can be incorporated into pharmaceutical compositions, which optionally include pharmaceutically acceptable carriers.

In another aspect, the present invention provides a method for detecting the presence of a PCIP nucleic acid molecule, protein or polypeptide in a biological sample by contacting the biological sample with an agent capable of detecting a PCIP nucleic acid molecule, protein or polypeptide such that the presence of a PCIP nucleic acid molecule, protein or polypeptide is detected in the biological sample.

In another aspect, the present invention provides a method for detecting the presence of PCIP activity in a biological sample by contacting the biological sample

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with an agent capable of detecting an indicator of PCIP activity such that the presence of PCIP activity is detected in the biological sample.

In another aspect, the invention provides a method for modulating PCIP activity comprising contacting a cell capable of expressing PCIP with an agent that modulates
5 PCIP activity such that PCIP activity in the cell is modulated. In one embodiment, the agent inhibits PCIP activity. In another embodiment, the agent stimulates PCIP activity. In one embodiment, the agent is an antibody that specifically binds to a PCIP protein. In another embodiment, the agent modulates expression of PCIP by modulating transcription of a PCIP gene or translation of a PCIP mRNA. In yet another
10 embodiment, the agent is a nucleic acid molecule having a nucleotide sequence that is antisense to the coding strand of a PCIP mRNA or a PCIP gene.

In one embodiment, the methods of the present invention are used to treat a subject having a disorder characterized by aberrant PCIP protein or nucleic acid expression or activity by administering an agent which is a PCIP modulator to the
15 subject. In one embodiment, the PCIP modulator is a PCIP protein. In another embodiment the PCIP modulator is a PCIP nucleic acid molecule. In yet another embodiment, the PCIP modulator is a peptide, peptidomimetic, or other small molecule. In a preferred embodiment, the disorder characterized by aberrant PCIP protein or nucleic acid expression is a CNS disorder or a cardiovascular disorder.

20 The present invention also provides a diagnostic assay for identifying the presence or absence of a genetic alteration characterized by at least one of (i) aberrant modification or mutation of a gene encoding a PCIP protein; (ii) mis-regulation of the gene; and (iii) aberrant post-translational modification of a PCIP protein, wherein a wild-type form of the gene encodes a protein with a PCIP activity.

25 In another aspect the invention provides a method for identifying a compound that binds to or modulates the activity of a PCIP protein, by providing an indicator composition comprising a PCIP protein having PCIP activity, contacting the indicator composition with a test compound, and determining the effect of the test compound on PCIP activity in the indicator composition to identify a compound that modulates the
30 activity of a PCIP protein.

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Other features and advantages of the invention will be apparent from the following detailed description and claims.

Brief Description of the Drawings

5 *Figure 1* depicts the cDNA sequence and predicted amino acid sequence of human 1v. The nucleotide sequence corresponds to nucleic acids 1 to 1463 of SEQ ID NO:1. The amino acid sequence corresponds to amino acids 1 to 216 of SEQ ID NO:2.

Figure 2 depicts the cDNA sequence and predicted amino acid sequence of rat 1v. The nucleotide sequence corresponds to nucleic acids 1 to 1856 of SEQ ID NO:3.
10 The amino acid sequence corresponds to amino acids 1 to 245 of SEQ ID NO:4.

Figure 3 depicts the cDNA sequence and predicted amino acid sequence of mouse 1v. The nucleotide sequence corresponds to nucleic acids 1 to 1907 of SEQ ID NO:5. The amino acid sequence corresponds to amino acids 1 to 216 of SEQ ID NO:6.

Figure 4 depicts the cDNA sequence and predicted amino acid sequence of rat 15 1vl. The nucleotide sequence corresponds to nucleic acids 1 to 1534 of SEQ ID NO:7. The amino acid sequence corresponds to amino acids 1 to 227 of SEQ ID NO:8.

Figure 5 depicts the cDNA sequence and predicted amino acid sequence of mouse 1vl. The nucleotide sequence corresponds to nucleic acids 1 to 1540 of SEQ ID NO:9. The amino acid sequence corresponds to amino acids 1 to 227 of SEQ ID NO:10.

20 *Figure 6* depicts the cDNA sequence and predicted amino acid sequence of rat 1vn. The nucleotide sequence corresponds to nucleic acids 1 to 955 of SEQ ID NO:11. The amino acid sequence corresponds to amino acids 1 to 203 of SEQ ID NO:12.

Figure 7 depicts the cDNA sequence and predicted amino acid sequence of human 9ql. The nucleotide sequence corresponds to nucleic acids 1 to 2009 of SEQ ID
25 NO:13. The amino acid sequence corresponds to amino acids 1 to 270 of SEQ ID NO:14.

Figure 8 depicts the cDNA sequence and predicted amino acid sequence of rat 9ql. The nucleotide sequence corresponds to nucleic acids 1 to 1247 of SEQ ID NO:15. The amino acid sequence corresponds to amino acids 1 to 257 of SEQ ID NO:16.

30 *Figure 9* depicts the cDNA sequence and predicted amino acid sequence of mouse 9ql. The nucleotide sequence corresponds to nucleic acids 1 to 2343 of SEQ ID

NO:17. The amino acid sequence corresponds to amino acids 1 to 270 of SEQ ID
NO:18.

Figure 10 depicts the cDNA sequence and predicted amino acid sequence of
human 9qm. The nucleotide sequence corresponds to nucleic acids 1 to 1955 of SEQ ID

5 NO:19. The amino acid sequence corresponds to amino acids 1 to 252 of SEQ ID
NO:20.

Figure 11 depicts the cDNA sequence and predicted amino acid sequence of rat
9qm. The nucleotide sequence corresponds to nucleic acids 1 to 2300 of SEQ ID

NO:21. The amino acid sequence corresponds to amino acids 1 to 252 of SEQ ID
10 NO:22.

Figure 12 depicts the cDNA sequence and predicted amino acid sequence of
human 9qs. The nucleotide sequence corresponds to nucleic acids 1 to 1859 of SEQ ID

NO:23. The amino acid sequence corresponds to amino acids 1 to 220 of SEQ ID
NO:24.

15 *Figure 13* depicts the cDNA sequence and predicted amino acid sequence of
monkey 9qs. The nucleotide sequence corresponds to nucleic acids 1 to 2191 of SEQ ID
NO:25. The amino acid sequence corresponds to amino acids 1 to 220 of SEQ ID
NO:26.

Figure 14 depicts the cDNA sequence and predicted amino acid sequence of rat
20 9qc. The nucleotide sequence corresponds to nucleic acids 1 to 2057 of SEQ ID NO:27.
The amino acid sequence corresponds to amino acids 1 to 252 of SEQ ID NO:28.

Figure 15 depicts the cDNA sequence and predicted amino acid sequence of rat
8t. The nucleotide sequence corresponds to nucleic acids 1 to 1904 of SEQ ID NO:29.
The amino acid sequence corresponds to amino acids 1 to 225 of SEQ ID NO:30.

25 *Figure 16* depicts the cDNA sequence and predicted amino acid sequence of
human p19. The nucleotide sequence corresponds to nucleic acids 1 to 619 of SEQ ID
NO:31. The amino acid sequence corresponds to amino acids 1 to 200 of SEQ ID
NO:32.

Figure 17 depicts the cDNA sequence and predicted amino acid sequence of rat
30 p19. The nucleotide sequence corresponds to nucleic acids 1 to 442 of SEQ ID NO:33.
The amino acid sequence corresponds to amino acids 1 to 109 of SEQ ID NO:34.

Figure 18 depicts the cDNA sequence and predicted amino acid sequence of mouse p19. The nucleotide sequence corresponds to nucleic acids 1 to 2644 of SEQ ID NO:35. The amino acid sequence corresponds to amino acids 1 to 256 of SEQ ID NO:36.

5 *Figure 19* depicts the cDNA sequence and predicted amino acid sequence of human W28559. The nucleotide sequence corresponds to nucleic acids 1 to 380 of SEQ ID NO:37. The amino acid sequence corresponds to amino acids 1 to 126 of SEQ ID NO:38.

10 *Figure 20* depicts the cDNA sequence and predicted amino acid sequence of human P193. The nucleotide sequence corresponds to nucleic acids 1 to 2176 of SEQ ID NO:39. The amino acid sequence corresponds to amino acids 1 to 41 of SEQ ID NO:40.

Figure 21 depicts a schematic representation of the rat 1v, the rat 9qm, and the mouse P19 proteins, aligned to indicate the conserved domains among these proteins.

15 *Figure 22* depicts the genomic DNA sequence of human 9q. *Figure 22A* depicts exon 1 and its flanking intron sequences (SEQ ID NO:46). *Figure 22B* depicts exons 2-11 and the flanking intron sequences (SEQ ID NO:47).

Figure 23 depicts the cDNA sequence and predicted amino acid sequence of monkey KChIP4a. The nucleotide sequence corresponds to nucleic acids 1 to 2413 of SEQ ID NO:48. The amino acid sequence corresponds to amino acids 1 to 233 of SEQ ID NO:49.

20 *Figure 24* depicts the cDNA sequence and predicted amino acid sequence of monkey KChIP4b. The nucleotide sequence corresponds to nucleic acids 1 to 1591 of SEQ ID NO:50. The amino acid sequence corresponds to amino acids 1 to 233 of SEQ ID NO:51.

Figure 25 depicts an alignment of KChIP4a, KChIP4b, 9ql, 1v, p19, and related human paralog (hsncspara) W28559. Amino acids identical to the consensus are shaded in black, conserved amino acids are shaded in gray.

25 *Figure 26* depicts the cDNA sequence and predicted amino acid sequence of rat 33b07. The nucleotide sequence corresponds to nucleic acids 1 to 2051 of SEQ ID

NO:52. The amino acid sequence corresponds to amino acids 1 to 407 of SEQ ID NO:53.

Figure 27 depicts the cDNA sequence and predicted amino acid sequence of human 33b07. The nucleotide sequence corresponds to nucleic acids 1 to 4148 of SEQ ID NO:54. The amino acid sequence corresponds to amino acids 1 to 414 of SEQ ID NO:55.

Figure 28 depicts the cDNA sequence and predicted amino acid sequence of rat 1p. The nucleotide sequence corresponds to nucleic acids 1 to 2643 of SEQ ID NO:56. The amino acid sequence corresponds to amino acids 1 to 267 of SEQ ID NO:57.

10 *Figure 29* depicts the cDNA sequence and predicted amino acid sequence of rat 7s. The nucleotide sequence corresponds to nucleic acids 1 to 2929 of SEQ ID NO:58. The amino acid sequence corresponds to amino acids 1 to 270 of SEQ ID NO:59.

Figure 30 depicts the cDNA sequence and predicted amino acid sequence of rat 29x. The nucleotide sequence corresponds to nucleic acids 1 to 1489 of SEQ ID NO:60. 15 The amino acid sequence corresponds to amino acids 1 to 351 of SEQ ID NO:61.

Figure 31 depicts the cDNA sequence of rat 25r. The nucleotide sequence corresponds to nucleic acids 1 to 1194 of SEQ ID NO:62.

Figure 32 depicts the cDNA sequence and predicted amino acid sequence of rat 5p. The nucleotide sequence corresponds to nucleic acids 1 to 600 of SEQ ID NO:63. 20 The amino acid sequence corresponds to amino acids 1 to 95 of SEQ ID NO:64.

Figure 33 depicts the cDNA sequence and predicted amino acid sequence of rat 7q. The nucleotide sequence corresponds to nucleic acids 1 to 639 of SEQ ID NO:65. The amino acid sequence corresponds to amino acids 1 to 212 of SEQ ID NO:66.

Figure 34 depicts the cDNA sequence and predicted amino acid sequence of rat 25 19r. The nucleotide sequence corresponds to nucleic acids 1 to 816 of SEQ ID NO:67. The amino acid sequence corresponds to amino acids 1 to 271 of SEQ ID NO:68.

Figure 35 depicts the cDNA sequence and predicted amino acid sequence of monkey KChIP4c. The nucleotide sequence corresponds to nucleic acids 1 to 2263 of SEQ ID NO:69. The amino acid sequence corresponds to amino acids 1 to 229 of SEQ ID NO:70. 30

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Figure 36 depicts the cDNA sequence and predicted amino acid sequence of monkey KChIP4d. The nucleotide sequence corresponds to nucleic acids 1 to 2259 of SEQ ID NO:71. The amino acid sequence corresponds to amino acids 1 to 250 of SEQ ID NO:72.

5 *Figure 37* depicts an alignment of KChIP4a, KChIP4b, KChIP4c, and KChIP4d.

Figure 38 depicts a graph showing the current traces from CHO cells which express Kv4.2 with or without KChIP2 (9ql). Cells are voltage clamped at -80 mV and stepped from -60 mV to +50 mV for 200ms. Peak current amplitudes at the various test voltages are shown in the right panel. *Figure 38* further depicts a table showing the
10 amplitude and kinetic effects of KChIP2 (9ql) on Kv4.2. KChIP2 expression alters the peak current amplitude, inactivation and recovery from inactivation time constants, and activation $V_{1/2}$.

Figure 39 depicts a graph showing the current traces from CHO cells which express Kv4.2 with or without KChIP3 (p19). Cells are voltage clamped at -80 mV and
15 stepped from -60 mV to +50 mV for 200ms. Peak current amplitudes at the various test voltages are shown in the right panel. *Figure 39* further depicts a table showing the amplitude and kinetic effects of KChIP3 (p19) on Kv4.2. KChIP3 causes alterations in peak current and inactivation and recovery from inactivation time constants.

Figure 40 depicts results from electrophysiological experiments demonstrating
20 that coexpression of KChIP1 dramatically alters the current density and kinetics of Kv4.2 channels expressed in CHO cells.

Figure 40A depicts current traces from a Kv4.2 transfected CHO cell. Current was evoked by depolarizing the cell sequentially from a holding potential of -80 mV to test potentials from -60 to 50 mV. Current traces are leak subtracted using a p/5
25 protocol. The current axis is shown at the same magnification as in (b) to emphasize the change in current amplitudes. Inset- Single current trace at 50mV at an expanded current axis to show the kinetics of current activation and inactivation.

Figure 40B depicts current traces as in (a), but from a cell transfected with equal amounts of DNA for Kv4.2 and KChIP1.

30 *Figure 40C* depicts peak current amplitude at all voltages from cells transfected with Kv4.2 alone (n=11) or cotransfected with KChIP1 (n=9).

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Figures 40D and 40E depict recovery from inactivation using a two pulse protocol. Kv4.2 alone (D) or coexpressed with KChIP1 (E) is driven into the inactivated state using a first pulse to 50 mV, then a second pulse to 50 mV is applied at varying times after the first pulse. Holding potential is -80 mV before and after all pulses.

5 *Figure 40F* depicts a summary of the percentage the peak current recovers between pulses for Kv4.2 (n=8) and Kv4.2 plus KChIP1 (n=5) transfected cells. The time constant of recovery from inactivation is fit to a single exponential.

10 *Figure 41* depicts an alignment of human KChIP family members with closely related members of the recoverin family of Ca²⁺ sensing proteins. (HIP:human hippocalcin; NCS1:rat neuronal calcium sensor 1). The alignment was performed using the MegAlign program for Macintosh (version 4.00 from DNASTAR) using the Clustal method with the PAM250 residue weight table and default parameters, and shaded using BOXSHADES. Residues identical to the consensus are shaded black, conservative substitutions are shaded grey. X, Y, Z and -X, -Y, -Z denote the positions of residues
15 which are responsible for binding to the calcium ion in the EF hand.

Figure 42 depicts a physical map of the IOSCA region.

Figure 43 depicts a linkage map showing the location of h9q and known markers associating with IOSCA and epilepsy.

20 **Detailed Description of the Invention**

The present invention is based, at least in part, on the discovery of novel nucleic acid molecules which encode gene products that interact with potassium channel proteins or possess substantial homology to the gene products of the invention that interact with potassium channel proteins (paralogs). Potassium channel proteins are, for
25 example, potassium channels having a Kv4.2 or Kv4.3 subunit. The nucleic acid molecules of the invention and their gene products are referred to herein as "Potassium Channel Interacting Proteins", "PCIP", or "KChIP" nucleic acid and protein molecules. Preferably, the PCIP proteins of the present invention interact with, *e.g.*, bind to a potassium channel protein, modulate the activity of a potassium channel protein, and/or
30 modulate a potassium channel mediated activity in a cell, *e.g.*, a neuronal or cardiac cell.

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As used herein, the term "PCIP family" when referring to the protein and nucleic acid molecules of the invention is intended to mean two or more proteins or nucleic acid molecules having a PCIP activity as defined herein. Such PCIP family members can be naturally or non-naturally occurring and can be from either the same or different species.

5 For example, a PCIP family can contain a first protein of human origin, as well as other, distinct proteins of human origin or alternatively, can contain homologues of non-human origin.

As used interchangeably herein, a "PCIP activity", "biological activity of PCIP" or "functional activity of PCIP", refers to an activity exerted by a PCIP protein,

10 polypeptide or nucleic acid molecule on a PCIP responsive cell or on a PCIP protein substrate, as determined *in vivo*, or *in vitro*, according to standard techniques. In one embodiment, a PCIP activity is a direct activity, such as an association with a PCIP-target molecule. As used herein, a "target molecule" or "binding partner" is a molecule with which a PCIP protein binds or interacts in nature, such that PCIP-mediated function

15 is achieved. A PCIP target molecule can be a non-PCIP molecule or a PCIP protein or polypeptide of the present invention. In an exemplary embodiment, a PCIP target molecule is a PCIP ligand. Alternatively, a PCIP activity is an indirect activity, such as a cellular signaling activity mediated by interaction of the PCIP protein with a PCIP ligand. The biological activities of PCIP are described herein.

20 For example, the PCIP proteins of the present invention can have one or more of the following activities: (1) they can interact with (*e.g.*, bind to) a potassium channel protein or portion thereof; (2) they can regulate the phosphorylation state of a potassium channel protein or portion thereof; (3) they can associate with (*e.g.*, bind) calcium and can, for example, act as calcium dependent kinases, *e.g.*, phosphorylate a potassium

25 channel or a G-protein coupled receptor in a calcium-dependent manner; (4) they can associate with (*e.g.*, bind) calcium and can, for example, act in a calcium-dependent manner in cellular processes, *e.g.*, act as calcium dependent transcription factors; (5) they can modulate a potassium channel mediated activity in a cell (*e.g.*, a neuronal cell such as a sensory neuron cell or a motor neuron cell, or a cardiac cell) to, for example,

30 beneficially affect the cell; (6) they can modulate chromatin formation in a cell, *e.g.*, a neuronal or cardiac cell; (7) they can modulate vesicular traffic and protein transport in a

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cell, *e.g.*, a neuronal or cardiac cell; (8) they can modulate cytokine signaling in a cell, *e.g.*, a neuronal or cardiac cell; (9) they can regulate the association of a potassium channel protein or portion thereof with the cellular cytoskeleton; (10) they can modulate cellular proliferation; (11) they can modulate the release of neurotransmitters; (12) they can modulate membrane excitability; (13) they can influence the resting potential of membranes; (14) they can modulate wave forms and frequencies of action potentials; and (15) they can modulate thresholds of excitation.

As used herein, a "potassium channel" includes a protein or polypeptide that is involved in receiving, conducting, and transmitting signals in an excitable cell.

Potassium channels are typically expressed in electrically excitable cells, *e.g.*, neurons, cardiac, skeletal and smooth muscle, renal, endocrine, and egg cells, and can form heteromultimeric structures, *e.g.*, composed of pore-forming and cytoplasmic subunits. Examples of potassium channels include: (1) the voltage-gated potassium channels, (2) the ligand-gated potassium channels, and (3) the mechanically-gated potassium channels. For a detailed description of potassium channels, see Kandel E.R. *et al.*, Principles of Neural Science, second edition, (Elsevier Science Publishing Co., Inc., N.Y. (1985)), the contents of which are incorporated herein by reference. The PCIP proteins of the present invention have been shown to interact with, for example, potassium channels having a Kv4.3 subunit or a Kv4.2 subunit.

As used herein, a "potassium channel mediated activity" includes an activity which involves a potassium channel, *e.g.*, a potassium channel in a neuronal cell or a cardiac cell, associated with receiving, conducting, and transmitting signals in, for example, the nervous system or in the heart. Potassium channel mediated activities include release of neurotransmitters, *e.g.*, dopamine or norepinephrine, from cells, *e.g.*, neuronal or cardiac cells; modulation of resting potential of membranes, wave forms and frequencies of action potentials, and thresholds of excitation; and modulation of processes such as integration of sub-threshold synaptic responses and the conductance of back-propagating action potentials in, for example, neuronal cells or cardiac cells.

As the PCIP proteins of the present invention modulate potassium channel mediated activities, they may be useful as novel diagnostic and therapeutic agents for potassium channel associated disorders and/or nervous system related disorders.

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Moreover, the PCIP proteins of the present invention modulate Kv4 potassium channels, e.g., potassium channels having a Kv4.2 or Kv4.3 subunit, which underlie the voltage-gated K⁺ current known as I_{to} (transient outward current) in the mammalian heart (Kaab S. *et al.* (1998) *Circulation* 98(14):1383-93; Dixon J.E. *et al.* (1996) *Circulation Research* 79(4):659-68; Nerbonne JM (1998) *Journal of Neurobiology* 37(1):37-59; Barry D.M. *et al.* (1998) *Circulation Research* 83(5):560-7; Barry D.M. *et al.* (1996) *Annual Review of Physiology* 58:363-94. This current underlies the rapid repolarization of cardiac myocytes during an action potential. It also participates in the inter-beat interval by controlling the rate at which cardiac myocytes reach the threshold for firing a subsequent action potential.

This current is also known to be down regulated in patients with cardiac hypertrophy, resulting in prolongation of the cardiac action potential. In these patients, action potential prolongation is thought to produce changes in calcium load and calcium handling within the myocardium, which contributes to the progression of cardiac disease from hypertrophy to heart failure (Wickenden *et al.* (1998) *Cardiovascular Research* 37:312). Interestingly, several PCIPs of the present invention (e.g., 9ql, 9qm, 9qs, shown in SEQ ID NOs:13, 15, 17, 19, 21, 23, and 25) bind to and modulate potassium channels containing a Kv4.2 or Kv4.3 subunit and contain calcium binding EF-hand domains. Because of mutations in these PCIP genes, defects in the expression of these calcium-binding PCIP proteins themselves, or defects in the interaction between these PCIPs and Kv4.2 or Kv4.3 channels, might be expected to lead to decreases in KV4.3 or Kv4.3(I_m) currents in the myocardium, therapeutic agents that alter PCIP expression or modulate the interaction between these PCIPs and Kv4.2 or Kv4.3 may be extremely valuable agents to slow or prevent the progression of disease from hypertrophy to heart failure.

As used herein, a "potassium channel associated disorder" includes a disorder, disease or condition which is characterized by a misregulation of a potassium channel mediated activity. Potassium channel associated disorders can detrimentally affect conveyance of sensory impulses from the periphery to the brain and/or conductance of motor impulses from the brain to the periphery; integration of reflexes; interpretation of sensory impulses; and emotional, intellectual (e.g., learning and memory), or motor

processes. Potassium channel associated disorders can further detrimentally affect electrical impulses that stimulate the cardiac muscle fibers to contract. Examples of potassium channel associated disorders include nervous system related disorders, as well as cardiovascular disorders.

As used herein, a “nervous system related disorder” includes a disorder, disease or condition which affects the nervous system. Examples of potassium channel associated disorders and nervous system related disorders include cognitive disorders, e.g., memory and learning disorders, such as amnesia, apraxia, agnosia, amnesic dysnomia, amnesic spatial disorientation, Kluver-Bucy syndrome, Alzheimer's related memory loss (Eglen R.M. (1996) *Pharmacol. and Toxicol.* 78(2):59-68; Perry E.K. (1995) *Brain and Cognition* 28(3):240-58) and learning disability; disorders affecting consciousness, e.g., visual hallucinations, perceptual disturbances, or delirium associated with Lewy body dementia; schizo-affective disorders (Dean B. (1996) *Mol. Psychiatry* 1(1):54-8), schizophrenia with mood swings (Bymaster F.P. (1997) *J. Clin. Psychiatry* 58 (suppl.10):28-36; Yeomans J.S. (1995) *Neuropharmacol.* 12(1):3-16; Reimann D. (1994) *J. Psychiatric Res.* 28(3):195-210), depressive illness (primary or secondary); affective disorders (Janowsky D.S. (1994) *Am. J. Med. Genetics* 54(4):335-44); sleep disorders (Kimura F. (1997) *J. Neurophysiol.* 77(2):709-16), e.g., REM sleep abnormalities in patients suffering from, for example, depression (Riemann D. (1994) *J. Psychosomatic Res.* 38 Suppl. 1:15-25; Bourgin P. (1995) *Neuroreport* 6(3): 532-6), paradoxical sleep abnormalities (Sakai K. (1997) *Eur. J. Neuroscience* 9(3):415-23), sleep-wakefulness, and body temperature or respiratory depression abnormalities during sleep (Shuman S.L. (1995) *Am. J. Physiol.* 269(2 Pt 2):R308-17; Mallick B.N. (1997) *Brain Res.* 750(1-2):311-7). Other examples of nervous system related disorders include disorders affecting pain generation mechanisms, e.g., pain related to irritable bowel syndrome (Mitch C.H. (1997) *J. Med. Chem.* 40(4):538-46; Shannon H.E. (1997) *J. Pharmac. and Exp. Therapeutics* 281(2):884-94; Bouaziz H. (1995) *Anesthesia and Analgesia* 80(6):1140-4; or Guimaraes A.P. (1994) *Brain Res.* 647(2):220-30) or chest pain; movement disorders (Monassi C.R. (1997) *Physiol. and Behav.* 62(1):53-9), e.g., Parkinson's disease related movement disorders (Finn M. (1997) *Pharmacol. Biochem. & Behavior* 57(1-2):243-9; Mayorga A.J. (1997) *Pharmacol. Biochem. & Behavior*

56(2):273-9); eating disorders, *e.g.*, insulin hypersecretion related obesity (Maccario M. (1997) *J. Endocrinol. Invest.* 20(1):8-12; Premawardhana L.D. (1994) *Clin. Endocrinol.* 40(5): 617-21); drinking disorders, *e.g.*, diabetic polydipsia (Murzi E. (1997) *Brain Res.* 752(1-2):184-8; Yang X. (1994) *Pharmacol. Biochem. & Behavior* 49(1):1-6);

5 neurodegenerative disorders, *e.g.*, Alzheimer's disease, dementias related to Alzheimer's
disease (such as Pick's disease), Parkinson's and other Lewy diffuse body diseases,
multiple sclerosis, amyotrophic lateral sclerosis, progressive supranuclear palsy,
epilepsy, spinocerebellar ataxia, epileptic syndromes, and Jakob-Creutzfeldt disease;
psychiatric disorders, *e.g.*, depression, schizophrenic disorders, Korsakoff's psychosis,
10 mania, anxiety disorders, bipolar affective disorders, or phobic disorders; neurological
disorders, *e.g.*, migraine; spinal cord injury; stroke; and head trauma.

As used herein, "epilepsy" includes a common neurological disorder caused by disturbances in the normal electrical functions of the brain. In normal brain function millions of tiny electrical charges pass from nerve cells in the brain to all parts of the body. In patients with epilepsy, this normal pattern is interrupted by sudden and unusually intense bursts of electrical energy, which may briefly affect a person's consciousness, bodily movements, or sensations. These physical changes are called epileptic seizures. There are two categories of seizures: partial seizures, which occur in one area of the brain, and generalized seizures, which affect nerve cells throughout the brain. Epilepsy may result from a brain injury before, during, or after birth; head trauma; poor nutrition; some infectious diseases; brain tumors; and some poisons. However, in many cases the cause is unknown. Attacks of epilepsy may be preceded by a feeling of unease or sensory discomfort called an aura, which indicates the beginning of the seizure. Signs of an impending epileptic seizure, which vary among patients, may include visual phenomena such as flickering lights or "sunbursts." Recently, a genetic linkage for epilepsy has been found on chromosome 10q, near marker D10S192: 10q22-q24 (Ottman et al. (1995) *Nature Genetics* 10:56-60). The many forms of epilepsy include: grand mal, Jacksonian, myoclonic progressive familial, petit mal, Lennox-Gastaut syndrome, febrile seizures, psycho-motor, and temporal lobe. The observations described herein are particularly useful in developing treatments for partial epilepsy.

As used herein, "ataxia" includes a common neurological disorder caused by disturbances in the normal electrical functions of the brain. Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant disorder which is genetically linked to the short arm of chromosome 6 based on linkage to the human major histocompatibility complex (HLA). See, for example, H. Yakura *et al.* (1974) *N. Engl. J. Med.*, 291, 154-155; and J. F. Jackson *et al.* (1977) *N. Engl. J. Med* 296, 1138-1141. SCA1 has been shown to be tightly linked to the marker D6S89 on the short arm of chromosome 6, telomeric to HLA. See, for example, L. P. W. Ranum *et al.*, *Am. J. Hum. Genet.*, 49, 31-41 (1991); and H. Y. Zoghbi *et al.*, *Am. J. Hum. Genet.*, 49, 23-30 (1991). The observations described herein are particularly useful in developing treatments for infantile onset spinocerebellar ataxia (IOSCA).

As used herein, a "cardiovascular disorder" includes a disorder affecting the cardiovascular system, *e.g.*, the heart. Examples of cardiovascular disorders include arteriosclerosis, ischemia reperfusion injury, restenosis, arterial inflammation, vascular wall remodeling, ventricular remodeling, rapid ventricular pacing, coronary microembolism, tachycardia, bradycardia, pressure overload, aortic bending, coronary artery ligation, vascular heart disease, atrial fibrillation, long-QT syndrome, congestive heart failure, sinus node dysfunction, angina, heart failure, hypertension, atrial fibrillation, atrial flutter, dilated cardiomyopathy, idiopathic cardiomyopathy, myocardial infarction, coronary artery disease, coronary artery spasm, or arrhythmia. In a preferred embodiment, the cardiovascular disorder is associated with an abnormal I_{to} current.

Some members of a PCIP family may also have common structural characteristics, such as a common structural domain or motif or a sufficient amino acid or nucleotide sequence homology as defined herein. Such PCIP family members can be naturally or non-naturally occurring and can be from either the same or different species. For example, a PCIP family can contain a first protein of human origin, as well as other, distinct proteins of human origin or alternatively, can contain homologues of non-human origin.

For example, members of a PCIP family which have common structural characteristics, may comprise at least one "calcium binding domain". As used herein,

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the term "calcium binding domain" includes an amino acid domain, *e.g.*, an EF hand (Baimbridge K.G. *et al.* (1992) *TINS* 15(8): 303-308), which is involved in calcium binding. Preferably, a calcium binding domain has a sequence, which is substantially identical to the consensus sequence:

5

EO••OO••ODKDGDG•O•••EF••OO. (SEQ ID NO:41).

O can be I, L, V or M, and "•" indicates a position with no strongly preferred residue. Each residue listed is present in more than 25% of sequences, and those underlined are present in more than 80% of sequences. Amino acid residues 126-154 and 174-202 of the human 1v protein, amino acid residues 126-154 and 174-202 of the rat 1v protein, amino acid residues 137-165 and 185-213 of the rat 1vl protein, amino acid residues 142-170 of the rat 1vn protein, amino acid residues 126-154 and 174-202 of the mouse 1v protein, amino acid residues 137-165 and 185-213 of the mouse 1vl protein, amino acid residues 144-172, 180-208, and 228-256 of the human 9q1 protein, amino acid residues 126-154, 162-190, and 210-238 of the human 9qm protein, amino acid residues 94-122, 130-158, and 178-206 of the human 9qs protein, amino acid residues 126-154, 162-190, and 210-238 of the rat 9qm protein, amino acid residues 131-159, 167-195, and 215-243 of the rat 9ql protein, amino acid residues 126-154, 162-190, and 210-238 of the rat 9qc protein, amino acid residues 99-127, 135-163, and 183-211 of the rat 8t protein, amino acid residues 144-172, 180-208, and 228-256 of the mouse 9ql protein, amino acid residues 94-122, 130-158, and 178-206 of the monkey 9qs protein, amino acid residues 94-122, 130-158, and 178-206 of the human p19 protein, amino acid residues 19-47 and 67-95 of the rat p19 protein, and amino acid residues 130-158, 166-194, and 214-242 of the mouse p19 protein comprise calcium binding domains (EF hands) (see Figure 21). Amino acid residues 116-127 and 152-163 of the monkey KChIP4a and KChIP4b proteins comprise calcium binding domains.

In another embodiment, the isolated PCIP proteins of the present invention are identified based on the presence of at least one conserved carboxyl-terminal domain which includes an amino acid sequence of about 100-200 amino acid residues in length, preferably 150-200 amino acid residues in length, and more preferably 185 amino acid

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residues in length, and which includes three EF hands. PCIP proteins of the present invention preferably contain a carboxyl-terminal domain which is at least about 70%, 71%, 74%, 75%, 76%, 80%, or more identical to the carboxyl terminal 185 amino acid residues of rat 1v, rat 9q, or mouse p19 (see Figures 21, 25, and 41).

5 Members of the PCIP family which also have common structural characteristics are listed in Table I and described below. The invention provides full length human, mouse, and rat 1v cDNA clones, full length mouse and rat cDNA clones of 1v splice variant 1vl, a partial rat cDNA clone of 1v splice variant 1vn, and the proteins encoded by these cDNAs. The invention further provides full length human and mouse and
10 partial rat 9ql cDNA clones, full length human and rat cDNA clones of 9ql splice variant 9qm, full length human and monkey cDNA clones of 9ql splice variant 9qs, a full length rat cDNA clone of 9ql splice variant 9qc, a partial rat cDNA clone of 9ql splice variant 8t, and the proteins encoded by these cDNAs. The invention also provides full length mouse and human and partial rat p19 cDNA clones and the proteins encoded by these
15 cDNAs. A full length human cDNA clone of p19 is provided, and a partial clone p193, representing the 3' end of the human p19 cDNA. In addition, the invention provides a partial human W28559 cDNA clone and the protein encoded by this cDNA. The invention further provides a full length monkey clone, KChIP4a, and a corresponding full length splice variant, KChIP4b and the proteins encoded by these cDNAs.

20 Other members of the PCIP family, *e.g.*, members of the PCIP family which do not have common structural characteristics, are listed in Table II and are described below. The present invention provides a full length human and a partial length rat 33b07 clone and the proteins encoded by these cDNAs. The present invention further provides partial length rat 1p clone and the protein encoded by this cDNA. In addition,
25 the present invention provides a partial length rat 7s clone and the protein encoded by this cDNA.

 The present invention further provides PCIP family members which represent previously identified cDNAs (29x, 25r, 5p, 7q, and 19r). These previously identified cDNAs are identified herein as PCIP family members, *i.e.*, as molecules which have a
30 PCIP activity, as described herein. Accordingly, the present invention provides methods for using these previously identified cDNAs, *e.g.*, methods for using these cDNAs in the

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screening assays, the diagnostic assays, the prognostic assays, and the methods of treatment described herein.

The PCIP molecules of the present invention were initially identified based on their ability, as determined using yeast two-hybrid assays (described in detail in Example 1), to interact with the amino-terminal 180 amino acids of rat Kv4.3 subunit. Further binding studies with other potassium subunits were performed to demonstrate specificity of the PCIP for Kv4.3 and Kv4.2. *In situ* localization, immuno-histochemical methods, co-immunoprecipitation and patch clamping methods were then used to clearly demonstrate that the PCIPs of the present invention interact with and modulate the activity of potassium channels, particularly those comprising a 4.3 or 4.2 subunit.

Several novel human, mouse, monkey, and rat PCIP family members have been identified, referred to herein as 1v, 9q, p19, W28559, KChIP4, 33b07, 1p, and rat 7s proteins and nucleic acid molecules. The human, rat, and mouse cDNAs encoding the 1v polypeptide are represented by SEQ ID NOs:1, 3, and 5, and shown in Figures 1, 2, and 3, respectively. In the brain, 1v mRNA is highly expressed in neocortical and hippocampal interneurons, in the thalamic reticular nucleus and medial habenula, in basal forebrain and striatal cholinergic neurons, in the superior colliculus, and in cerebellar granule cells. The 1v polypeptide is highly expressed in the somata, dendrites, axons and axon terminals of cells that express 1v mRNA. Splice variants of the 1v gene have been identified in rat and mouse and are represented by SEQ ID NOs: 7, 9, and 11 and shown in Figures 4, 5, and 6, respectively. 1v polypeptide interacts with potassium channels comprising Kv4.3 or kv4.2 subunits, but not with Kv1.1 subunits. As determined by Northern blot, the 1v transcripts (mRNA) are expressed predominantly in the brain

The 8t cDNA (SEQ ID NO: 29) encodes a polypeptide having a molecular weight of approximately 26 kD corresponding to SEQ ID NO:30 (see Figure 15). The 8t polypeptide interacts with potassium channel comprising Kv4.3 or Kv4.2 subunits, but not with Kv1.1 subunits. As determined by Northern blot and *in situ* data, the 8t mRNA is expressed predominantly in the heart and the brain. The 8t cDNA is a splice variant of 9q.

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length. Rat 33b07 binds rKv4.3N and rKv4.2N with slight preference for rKv4.2N in yeast 2-hybrid assays.

The nucleotide sequence of the full length human 33b07 cDNA and the predicted amino acid sequence of the human 33b07 polypeptide are shown in Figure 27 and in
5 SEQ ID NOs:54 and 55, respectively.

The nucleotide sequence of the partial length rat 1p cDNA and the predicted amino acid sequence of the rat 1p polypeptide are shown in Figure 28 and in SEQ ID
10 NOs:56 and 57, respectively. The rat 1p cDNA encodes a protein having a molecular weight of approximately 28.6 kD and which is 267 amino acid residues in length. Rat 1p binds rKv4.3N and rKv4.2N with slight preference for rKv4.3N in yeast two-hybrid assays.

The nucleotide sequence of the partial length rat 7s cDNA and the predicted amino acid sequence of the rat 7s polypeptide are shown in Figure 29 and in SEQ ID
15 NOs:58 and 59, respectively. The rat 7s cDNA encodes a protein having a molecular weight of approximately 28.6 kD and which is 270 amino acid residues in length. Rat 7s binds rKv4.3N and rKv4.2N with preference for rKv4.3N in yeast two-hybrid assays.

The sequences of the present invention are summarized below, in Tables I and II.

20 **Table I**

Novel Polynucleotides and Polypeptides of the Present Invention (full length except where noted)

PCIP	Nucleic Acid Molecule Form	Source	SEQ ID NO: DNA	SEQ ID NO: PROTEIN	ATCC
1v or KChIP1	1v	human (225-875)*	1	2	98994
	1v	rat (210-860)	3	4	98946

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	1v	mouse (477-1127)	5	6	98945
	1vl	rat (31-714)	7	8	98942
	1vl	mouse (77-760)	9	10	98943
	1vn (partial)	rat (345-955)	11	12	98944
9q or KChIP2	Genomic DNA sequence (Exon 1 and flanking intron sequences)	human	46		
	Genomic DNA sequence (Exons 2-11 and flanking intron sequences)	human	47		
	9ql	human (207-1019)	13	14	98993 98991
	9ql (partial)	rat (2-775)	15	16	98948
	9ql	mouse (181 -993)	17	18	98937
	9qm	human (207-965)	19	20	98993 98991
	9qm	rat (214-972)	21	22	98941

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	9qs	human (207-869)	23	24	98951
	9qs	monkey (133-795)	25	26	98950
	9qc	rat (208-966)	27	28	98947
	8t (partial)	rat (1-678)	29	30	98939
p19 or KChIP3	p19	Human (1-771)	31	32	PTA-316
	p19 (partial)	rat (1-330)	33	34	98936
	p19	mouse (49-819)	35	36	98940
	p193 (partial)	Human (2-127)	39	40	98949
W28559	W28559 (partial)	human (1-339)	37	38	
KChIP4	KChIP4a	Monkey (265-966)	48	49	
	KChIP4b C-terminal splice variant	Monkey (265-966)	50	51	
	KChIP4c splice variant	Monkey (122-811)	69	70	
	KChIP4d splice variant	Monkey (64-816)	71	72	

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* The coordinates of the coding sequence are shown in parenthesis. The first column indicates the PCIPs which were identified and column 2 indicates the various nucleic acid forms identified for each PCIP.

5 Table II

Polynucleotides and Polypeptides of the Present Invention (full length except where noted)

PCIP	Nucleic Acid Molecule Form	Source	SEQ ID NO: DNA	SEQ ID NO: PROTEIN	ATCC
33b07 Novel	33b07	Human (88-1332)	52	53	PTA-316
	33b07	Rat (85-1308)	54	55	
1p Novel	1p (partial)	Rat (1-804)	56	57	
7s Novel	7s (partial)	Rat (1-813)	58	59	
29x	29x	Rat (433-1071)	60	61	
	25r splice variant of 29x	Rat (130-768)	62		
5p	5p	Rat (52-339)	63	64	
7q	7q	Rat (1-639)	65	66	
19r	19r	Rat (1-816)	67	68	

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* The coordinates of the coding sequence are shown in parenthesis. The first column indicates the four families of PCIPs which were identified and column 2 indicates the various nucleic acid forms identified for each family. Novel molecules are also indicated.

5 Plasmids containing the nucleotide sequences encoding human, rat and monkey PCIPs were deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on November 17, 1998, and assigned the Accession Numbers described above. These deposits will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of
10 Microorganisms for the Purposes of Patent Procedure. These deposits were made merely as a convenience for those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. §112.

 Clones containing cDNA molecules encoding human p19 (clone EphP19) and human 33b07 (clone Eph33b07) were deposited with American Type Culture Collection
15 (Manassas, VA) on July 8, 1998 as Accession Number PTA-316, as part of a composite deposit representing a mixture of two strains, each carrying one recombinant plasmid harboring a particular cDNA clone. (The ATCC strain designation for the mixture of hP19 and h33b07 is EphP19h33b07mix).

 To distinguish the strains and isolate a strain harboring a particular cDNA clone,
20 an aliquot of the mixture can be streaked out to single colonies on LB plates supplemented with 100 ug/ml ampicillin, single colonies grown, and then plasmid DNA extracted using a standard miniprep procedure. Next, a sample of the DNA miniprep can be digested with NotI and the resultant products resolved on a 0.8% agarose gel using standard DNA electrophoresis conditions. The digest gives the
25 following band patterns: EphP19: 7 kb 9 (single band), Eph33b07: 5.8 kb (single band).

 Various aspects of the invention are described in further detail in the following subsections:

I. Isolated Nucleic Acid Molecules

One aspect of the invention pertains to isolated nucleic acid molecules that encode PCIP proteins or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify PCIP-encoding nucleic acid molecules (*e.g.*, PCIP mRNA) and fragments for use as PCR primers for the amplification or mutation of PCIP nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA) and RNA molecules (*e.g.*, mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

An "isolated" nucleic acid molecule is one which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated PCIP nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or the nucleotide sequence of the DNA insert of the plasmid deposited with

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ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or portion of the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, as a hybridization probe, PCIP nucleic acid molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994 can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ

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5 nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as
Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944,
98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994.

10 according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to PCIP nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

comprises the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, or a portion of any of these nucleotide sequences.

25 In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID

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NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, or a portion of any of these nucleotide sequences. A nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, thereby forming a stable duplex.

In still another preferred embodiment, an isolated nucleic acid molecule of the present invention comprises a nucleotide sequence which is at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more identical to the entire length of the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or the entire length of the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, or a portion of any of these nucleotide sequences.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, for example a fragment which can be used as a probe or primer or a fragment encoding a biologically active portion of a PCIP protein. The nucleotide sequence determined from the cloning of the PCIP gene allows for the generation of probes and primers designed for use in identifying and/or cloning other PCIP family members, as well as PCIP homologues from other species.

30 The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes

under stringent conditions to at least about 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 consecutive nucleotides of a sense sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as

Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, of an anti-sense sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, or of a naturally occurring allelic variant or mutant of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994. In an exemplary embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which is 350-400,

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having a PCIP biological activity (the biological activities of the PCIP proteins are described herein), expressing the encoded portion of the PCIP protein (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the PCIP protein.

- 5 The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID
- 10 NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71 or the nucleotide sequence of the DNA insert of the plasmid deposited with 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, due to degeneracy of the
- 15 genetic code and thus encode the same PCIP proteins as those encoded by the nucleotide sequence shown in SSEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID
- 20 NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71 or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or
- 25 98994. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID
- 30 NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID

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NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72.

In addition to the PCIP nucleotide sequences shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the PCIP proteins may exist within a population (*e.g.*, the human population). Such genetic polymorphism in the PCIP genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which include an open reading frame encoding a PCIP protein, preferably a mammalian PCIP protein, and can further include non-coding regulatory sequences, and introns.

Allelic variants of human PCIP include both functional and non-functional PCIP proteins. Functional allelic variants are naturally occurring amino acid sequence variants of the human PCIP protein that maintain the ability to bind a PCIP ligand and/or modulate any of the PCIP activities described herein. Functional allelic variants will typically contain only conservative substitution of one or more amino acids of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72 or substitution, deletion or insertion of non-critical residues in non-critical regions of the protein.

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Non-functional allelic variants are naturally occurring amino acid sequence variants of the human PCIP protein that do not have the ability to either bind a PCIP ligand and/or modulate any of the PCIP activities described herein. Non-functional allelic variants will typically contain a non-conservative substitution, a deletion, or

5 insertion or premature truncation of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID

10 NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72 or a substitution, insertion or deletion in critical residues or critical regions.

The present invention further provides non-human orthologues of the human PCIP protein. Orthologues of the human PCIP protein are proteins that are isolated from non-human organisms and possess the same PCIP ligand binding and/or modulation of

15 potassium channel mediated activities of the human PCIP protein. Orthologues of the human PCIP protein can readily be identified as comprising an amino acid sequence that is substantially identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID

20 NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72.

Moreover, nucleic acid molecules encoding other PCIP family members and, thus, which have a nucleotide sequence which differs from the PCIP sequences of SEQ

25 ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID

30 NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936,

98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994 are intended to be within the scope of the invention. For example, another PCIP cDNA can be identified based on the nucleotide sequence of human PCIP. Moreover, nucleic acid molecules encoding PCIP

5 proteins from different species, and thus which have a nucleotide sequence which differs from the PCIP sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID

10 NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71 or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or

15 98994 are intended to be within the scope of the invention. For example, a mouse PCIP cDNA can be identified based on the nucleotide sequence of a human PCIP.

Nucleic acid molecules corresponding to natural allelic variants and homologues of the PCIP cDNAs of the invention can be isolated based on their homology to the PCIP nucleic acids disclosed herein using the cDNAs disclosed herein, or a portion

20 thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 15, 20, 25, 30 or more nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of

25 SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID

30 NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71 or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as

Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994. In other embodiment, the nucleic acid is at least 30, 50, 100, 150, 200, 250, 300, 307, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 949, or 950 nucleotides in length. As

5 used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% identical to each other typically remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% identical to each other typically

10 remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-limiting example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50°C, preferably at 55°C,

15 and more preferably at 60°C or 65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1 corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

20 In addition to naturally-occurring allelic variants of the PCIP sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID

25 NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938,

30 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949,

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98950, 98951, 98991, 98993, or 98994, thereby leading to changes in the amino acid sequence of the encoded PCIP proteins, without altering the functional ability of the PCIP proteins. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of PCIP (*e.g.*, the sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the PCIP proteins of the present invention, are predicted to be particularly unamenable to alteration. Furthermore, additional amino acid residues that are conserved between the PCIP proteins of the present invention and other members of the PCIP family of proteins are not likely to be amenable to alteration.

Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding PCIP proteins that contain changes in amino acid residues that are not essential for activity. Such PCIP proteins differ in amino acid sequence from SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID

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NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72, yet retain biological activity. In one embodiment, the

5 isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ

10 ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72.

An isolated nucleic acid molecule encoding a PCIP protein homologous to the

15 protein of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID

20 NO:59, SEQ ID NO:70, or SEQ ID NO:72 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID

25 NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947,

30 98948, 98949, 98950, 98951, 98991, 98993, or 98994, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations

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can be introduced into SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a PCIP protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a PCIP coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for PCIP biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID

NO:69, or SEQ ID NO:71, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

In a preferred embodiment, a mutant PCIP protein can be assayed for the ability to (1) interact with (*e.g.*, bind to) a potassium channel protein or portion thereof; (2) regulate the phosphorylation state of a potassium channel protein or portion thereof; (3) associate with (*e.g.*, bind) calcium and, for example, act as a calcium dependent kinase, *e.g.*, phosphorylate a potassium channel in a calcium-dependent manner; (4) associate with (*e.g.*, bind) calcium and, for example, act as a calcium dependent transcription factor; (5) modulate a potassium channel mediated activity in a cell (*e.g.*, a neuronal or cardiac cell) to, for example, beneficially affect the cell; (6) modulate the release of neurotransmitters; (7) modulate membrane excitability; (8) influence the resting potential of membranes; (9) modulate wave forms and frequencies of action potentials; and (10) modulate thresholds of excitation.

In addition to the nucleic acid molecules encoding PCIP proteins described above, another aspect of the invention pertains to isolated nucleic acid molecules which are antisense thereto. An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire PCIP coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding PCIP. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding PCIP. The term "noncoding region" refers to 5' and 3' sequences which flank

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the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding PCIP disclosed herein, antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of PCIP mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of PCIP mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of PCIP mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5- oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a

nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a PCIP protein to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention include direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-*o*-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes

(described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave PCIP mRNA transcripts to thereby inhibit translation of PCIP mRNA. A ribozyme having specificity for a PCIP-encoding nucleic acid can be designed based upon the nucleotide sequence of a PCIP cDNA disclosed herein (*i.e.*,
5 SEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID
10 NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the
15 nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a PCIP-encoding mRNA. See, *e.g.*, Cech *et al.* U.S. Patent No. 4,987,071; and Cech *et al.* U.S. Patent No. 5,116,742. Alternatively, PCIP mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.
20 Alternatively, PCIP gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the PCIP (*e.g.*, the PCIP promoter and/or enhancers) to form triple helical structures that prevent transcription of the PCIP gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992)
25 *Bioassays* 14(12):807-15.

In yet another embodiment, the PCIP nucleic acid molecules of the present invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to
30 generate peptide nucleic acids (see Hyrup B. *et al.* (1996) *Bioorganic & Medicinal Chemistry* 4 (1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs"

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refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *et al.* Proc. Natl. Acad. Sci. 93: 14670-675.

PNAs of PCIP nucleic acid molecules can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of PCIP nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (*e.g.*, by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (*e.g.*, S1 nucleases (Hyrup B. (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *supra*).

In another embodiment, PNAs of PCIP can be modified, (*e.g.*, to enhance their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of PCIP nucleic acid molecules can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, (*e.g.*, RNase H and DNA polymerases), to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup B. (1996) *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup B. (1996) *supra* and Finn P.J. *et al.* (1996) *Nucleic Acids Res.* 24 (17): 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used as a between the PNA and the 5' end of DNA (Mag, M. *et*

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5 *Bioorganic Med. Chem. Lett.* 5: 1119-11124).

10 PCT Publication No. W088/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication
No. W089/10134). In addition, oligonucleotides can be modified with hybridization-
triggered cleavage agents (See, *e.g.*, Krol *et al.* (1988) *Bio-Techniques* 6:958-976) or
intercalating agents. (See, *e.g.*, Zon (1988) *Pharm. Res.* 5:539-549). To this end, the
oligonucleotide may be conjugated to another molecule, (*e.g.*, a peptide, hybridization
15 triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

II. Isolated PCIP Proteins and Anti-PCIP Antibodies

20 to raise anti-PCIP antibodies. In one embodiment, native PCIP proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, PCIP proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a PCIP protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

25 An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the PCIP protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of PCIP protein in which
30 the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of

cellular material" includes preparations of PCIP protein having less than about 30% (by dry weight) of non-PCIP protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-PCIP protein, still more preferably less than about 10% of non-PCIP protein, and most preferably less than about 5% non-PCIP

5 protein. When the PCIP protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of PCIP protein in which the protein is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of PCIP protein having less than about 30% (by dry weight) of chemical precursors or non-PCIP chemicals, more preferably less than about 20% chemical precursors or non-PCIP chemicals, still more preferably less than about 10% chemical precursors or non-PCIP chemicals, and most preferably less than about 5% chemical precursors or non-PCIP chemicals.

As used herein, a "biologically active portion" of a PCIP protein includes a fragment of a PCIP protein which participates in an interaction between a PCIP molecule and a non-PCIP molecule. Biologically active portions of a PCIP protein include peptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequence of the PCIP protein, *e.g.*, the amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72, which include less amino acids than the full length PCIP proteins, and exhibit at least one activity of a PCIP protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the PCIP protein, *e.g.*, binding of a potassium channel subunit. A biologically active

portion of a PCIP protein can be a polypeptide which is, for example, 10, 25, 50, 100, 200, or more amino acids in length. Biologically active portions of a PCIP protein can be used as targets for developing agents which modulate a potassium channel mediated activity.

5 In one embodiment, a biologically active portion of a PCIP protein comprises at least one calcium binding domain.

It is to be understood that a preferred biologically active portion of a PCIP protein of the present invention may contain at least one of the above-identified structural domains. A more preferred biologically active portion of a PCIP protein may contain at least two of the above-identified structural domains. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native PCIP protein.

In a preferred embodiment, the PCIP protein has an amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72. In other embodiments, the PCIP protein is substantially homologous to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72, and retains the functional activity of the protein of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID

NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail in subsection I above. Accordingly, in another embodiment, the PCIP protein is a protein which comprises an amino acid sequence at least about 50%, 55%, 60%, 65%,
 5 70%, 75%, 80%, 85%, 90%, 95%, 98% or more identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID
 10 NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72.

Isolated proteins of the present invention, preferably 1v, 9q, p19, W28559, KChIP4a, KChIP4b, 33b07, 1p, or 7s proteins, have an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ
 15 ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72 or are encoded by a nucleotide
 20 sequence sufficiently identical to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID
 25 NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71. As used herein, the term "sufficiently identical" refers to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (*e.g.*, an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide
 30 sequences share common structural domains or motifs and/or a common functional activity. For example, amino acid or nucleotide sequences which share common structural domains

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have at least 30%, 40%, or 50% identity, preferably 60% identity, more preferably 70%-80%, and even more preferably 90-95% identity across the amino acid sequences of the domains and contain at least one and preferably two structural domains or motifs, are defined herein as sufficiently identical. Furthermore, amino acid or nucleotide sequences

5 which share at least 30%, 40%, or 50%, preferably 60%, more preferably 70-80%, or 90-95% identity and share a common functional activity are defined herein as sufficiently identical.

Preferred proteins are PCIP proteins having at least one calcium binding domain and, preferably, a PCIP activity. Other preferred proteins are PCIP proteins having at least

10 one calcium binding domain, and are, preferably, encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID

15 NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71.

To determine the percent identity of two amino acid sequences or of two nucleic

20 acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more

25 preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence (*e.g.*, when aligning a second sequence to the PCIP amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID

30 NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID

NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72 having 177 amino acid residues, at least 80, preferably at least 100, more preferably at least 120, even more preferably at least 140, and even more preferably at least 150, 160 or 170 amino acid residues are aligned). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blosum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0 or 2.0U), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J.*

Mol. Biol. 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to PCIP nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain

5 amino acid sequences homologous to PCIP protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

10 The invention also provides PCIP chimeric or fusion proteins. As used herein, a PCIP "chimeric protein" or "fusion protein" comprises a PCIP polypeptide operatively linked to a non-PCIP polypeptide. An "PCIP polypeptide" refers to a polypeptide having an amino acid sequence corresponding to PCIP, whereas a "non-PCIP polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a

15 protein which is not substantially homologous to the PCIP protein, *e.g.*, a protein which is different from the PCIP protein and which is derived from the same or a different organism. Within a PCIP fusion protein the PCIP polypeptide can correspond to all or a portion of a PCIP protein. In a preferred embodiment, a PCIP fusion protein comprises at least one biologically active portion of a PCIP protein. In another preferred

20 embodiment, a PCIP fusion protein comprises at least two biologically active portions of a PCIP protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the PCIP polypeptide and the non-PCIP polypeptide are fused in-frame to each other. The non-PCIP polypeptide can be fused to the N-terminus or C-terminus of the PCIP polypeptide.

25 For example, in one embodiment, the fusion protein is a GST-PCIP fusion protein in which the PCIP sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant PCIP.

In another embodiment, the fusion protein is a PCIP protein containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian

30 host cells), expression and/or secretion of PCIP can be increased through use of a heterologous signal sequence.

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The PCIP fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The PCIP fusion proteins can be used to affect the bioavailability of a PCIP substrate. Use of PCIP fusion proteins may be useful therapeutically for the treatment of potassium channel associated disorders such as CNS disorders, *e.g.*, neurodegenerative disorders such as Alzheimer's disease, dementias related to Alzheimer's disease (such as Pick's disease), Parkinson's and other Lewy diffuse body diseases, multiple sclerosis, amyotrophic lateral sclerosis, progressive supranuclear palsy, epilepsy, spinocerebellar ataxia, and Jakob-Creutzfeldt disease; psychiatric disorders, *e.g.*, depression, schizophrenic disorders, Korsakoff's psychosis, mania, anxiety disorders, or phobic disorders; learning or memory disorders, *e.g.*, amnesia or age-related memory loss; and neurological disorders; *e.g.*, migraine. Use of PCIP fusion proteins may also be useful therapeutically for the treatment of potassium channel associated disorders such as cardiovascular disorders, *e.g.*, arteriosclerosis, ischemia reperfusion injury, restenosis, arterial inflammation, vascular wall remodeling, ventricular remodeling, rapid ventricular pacing, coronary microembolism, tachycardia, bradycardia, pressure overload, aortic bending, coronary artery ligation, vascular heart disease, atrial fibrillation or congestive heart failure.

Moreover, the PCIP-fusion proteins of the invention can be used as immunogens to produce anti-PCIP antibodies in a subject, to purify PCIP ligands and in screening assays to identify molecules which inhibit the interaction of PCIP with a PCIP substrate.

Preferably, a PCIP chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene

fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel *et al.* John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). A PCIP-

5 encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the PCIP protein.

The present invention also pertains to variants of the PCIP proteins which function as either PCIP agonists (mimetics) or as PCIP antagonists. Variants of the PCIP proteins can be generated by mutagenesis, *e.g.*, discrete point mutation or
10 truncation of a PCIP protein. An agonist of the PCIP proteins can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of a PCIP protein. An antagonist of a PCIP protein can inhibit one or more of the activities of the naturally occurring form of the PCIP protein by, for example, competitively modulating a potassium channel mediated activity of a PCIP protein. Thus, specific
15 biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the PCIP protein.

In one embodiment, variants of a PCIP protein which function as either PCIP
20 agonists (mimetics) or as PCIP antagonists can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of a PCIP protein for PCIP protein agonist or antagonist activity. In one embodiment, a variegated library of PCIP variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of PCIP variants can be produced by, for
25 example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential PCIP sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of PCIP sequences therein. There are a variety of methods which can be used to produce libraries of potential PCIP variants from a degenerate
30 oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an

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appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential PCIP sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, e.g., Narang, S.A. (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu. Rev.*

- 5 *Biochem.* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucleic Acid Res.* 11:477.

In addition, libraries of fragments of a PCIP protein coding sequence can be used to generate a variegated population of PCIP fragments for screening and subsequent selection of variants of a PCIP protein. In one embodiment, a library of coding sequence
10 fragments can be generated by treating a double stranded PCR fragment of a PCIP coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with
15 S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal and internal fragments of various sizes of the PCIP protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA
20 libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of PCIP proteins. The most widely used techniques, which are amenable to high throughput analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting
25 library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify PCIP variants (Arkin and Yourvan
30 (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6(3):327-331).

In one embodiment, cell based assays can be exploited to analyze a variegated PCIP library. For example, a library of expression vectors can be transfected into a cell line which ordinarily possesses a potassium channel mediated activity. The effect of the PCIP mutant on the potassium channel mediated activity can then be detected, *e.g.*, by any of a number of enzymatic assays or by detecting the release of a neurotransmitter. Plasmid DNA can then be recovered from the cells which score for inhibition, or alternatively, potentiation of the potassium channel mediated activity, and the individual clones further characterized.

An isolated PCIP protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind PCIP using standard techniques for polyclonal and monoclonal antibody preparation. A full-length PCIP protein can be used or, alternatively, the invention provides antigenic peptide fragments of PCIP for use as immunogens. The antigenic peptide of PCIP comprises at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72 and encompasses an epitope of PCIP such that an antibody raised against the peptide forms a specific immune complex with PCIP. Preferably, the antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

Preferred epitopes encompassed by the antigenic peptide are regions of PCIP that are located on the surface of the protein, *e.g.*, hydrophilic regions, as well as regions with high antigenicity.

A PCIP immunogen typically is used to prepare antibodies by immunizing a suitable subject, (*e.g.*, rabbit, goat, mouse or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed PCIP protein or a chemically synthesized PCIP polypeptide. The preparation

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can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic PCIP preparation induces a polyclonal anti-PCIP antibody response.

Accordingly, another aspect of the invention pertains to anti-PCIP antibodies.

- 5 The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as PCIP. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')₂ fragments which can be generated by treating the
- 10 antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind PCIP. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of PCIP. A monoclonal antibody composition
- 15 thus typically displays a single binding affinity for a particular PCIP protein with which it immunoreacts.

- Polyclonal anti-PCIP antibodies can be prepared as described above by immunizing a suitable subject with a PCIP immunogen. The anti-PCIP antibody titer in the immunized subject can be monitored over time by standard techniques, such as with
- 20 an enzyme linked immunosorbent assay (ELISA) using immobilized PCIP. If desired, the antibody molecules directed against PCIP can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, *e.g.*, when the anti-PCIP antibody titers are highest, antibody-producing cells can be
 - 25 obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497) (see also, Brown *et al.* (1981) *J. Immunol.* 127:539-46; Brown *et al.* (1980) *J. Biol. Chem.* 255:4980-83; Yeh *et al.* (1976) *Proc. Natl. Acad. Sci. USA* 76:2927-31; and Yeh *et al.* (1982) *Int. J. Cancer* 29:269-75), the
 - 30 more recent human B cell hybridoma technique (Kozbor *et al.* (1983) *Immunol Today* 4:72), the EBV-hybridoma technique (Cole *et al.* (1985), *Monoclonal Antibodies and*

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Cancer Therapy, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally R. H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); E. A. Lerner (1981) *Yale J. Biol. Med.*, 54:387-402; M. L. Gefter *et al.* (1977) *Somatic Cell Genet.* 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a PCIP immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds PCIP.

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-PCIP monoclonal antibody (see, *e.g.*, G. Galfre *et al.* (1977) *Nature* 266:55052; Gefter *et al.* *Somatic Cell Genet.*, cited *supra*; Lerner, *Yale J. Biol. Med.*, cited *supra*; Kenneth, *Monoclonal Antibodies*, cited *supra*). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (*e.g.*, a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, *e.g.*, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind PCIP, *e.g.*, using a standard ELISA assay.

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Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-PCIP antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with PCIP to thereby isolate immunoglobulin library members that bind PCIP.

- 5 Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* PCT International Publication No. WO 92/18619; Dower *et al.* PCT International Publication No. WO 91/17271; Winter *et al.* PCT International Publication WO 92/20791; Markland *et al.* PCT International Publication No. WO 92/15679; Breitling *et al.* PCT International Publication WO 93/01288; McCafferty *et al.* PCT International Publication No. WO 92/01047; Garrard *et al.* PCT International Publication No. WO 92/09690; Ladner *et al.* PCT International Publication No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J. Mol. Biol.* 226:889-896; Clarkson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:3576-3580; Garrad *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc. Acid Res.* 19:4133-4137; Barbas *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:7978-7982; and McCafferty *et al.* *Nature* (1990) 348:552-554.
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- Additionally, recombinant anti-PCIP antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson *et al.* International Application No. PCT/US86/02269; Akira, *et al.* European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison *et al.* European Patent Application 173,494; Neuberger *et al.* PCT International Publication No. WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567;
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Cabilly *et al.* European Patent Application 125,023; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu *et al.* (1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci. USA*

84:214-218; Nishimura *et al.* (1987) *Canc. Res.* 47:999-1005; Wood *et al.* (1985)

5 *Nature* 314:446-449; and Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559);

Morrison, S. L. (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214;

Winter U.S. Patent 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyan *et al.*

(1988) *Science* 239:1534; and Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060.

An anti-PCIP antibody (*e.g.*, monoclonal antibody) can be used to isolate PCIP

by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-PCIP antibody can facilitate the purification of natural PCIP from cells and of recombinantly produced PCIP expressed in host cells. Moreover, an anti-PCIP antibody can be used to detect PCIP protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the PCIP protein. Anti-PCIP antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

III. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding a PCIP protein (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for

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example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cell and those which direct expression of the nucleotide sequence only in certain host cells (e.g.,
5 tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as
10 described herein (e.g., PCIP proteins, mutant forms of PCIP proteins, fusion proteins, and the like).

The recombinant expression vectors of the invention can be designed for expression of PCIP proteins in prokaryotic or eukaryotic cells. For example, PCIP proteins can be expressed in bacterial cells such as *E. coli*, insect cells (using
15 baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

20 Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein;
25 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and
30 their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B.

and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be utilized in PCIP activity assays, (*e.g.*, direct
5 assays or competitive assays described in detail below), or to generate antibodies specific for PCIP proteins, for example. In a preferred embodiment, a PCIP fusion protein expressed in a retroviral expression vector of the present invention can be utilized to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time
10 has passed (*e.g.*, six (6) weeks).

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression from the pTrc vector relies on host
15 RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5
20 promoter.

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another
25 strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.*, (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

30 In another embodiment, the PCIP expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari, *et*

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al., (1987) *Embo J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.*, (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and picZ (InVitrogen Corp, San Diego, CA).

Alternatively, PCIP proteins can be expressed in insect cells using baculovirus
 5 expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*, Sf 9 cells) include the pAc series (Smith *et al.* (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian
 10 expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both
 15 prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

In another embodiment, the recombinant mammalian expression vector is
 20 capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916),
 25 and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-

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regulated promoters are also encompassed, for example the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α -fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a
5 DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to PCIP mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct
10 the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a
15 high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. *et al.*, Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant
20 expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be
25 identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, a PCIP protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other
30 suitable host cells are known to those skilled in the art.

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Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (*Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989*), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding a PCIP protein or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) a PCIP protein. Accordingly, the invention further provides methods for producing a PCIP protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding a PCIP protein has been introduced) in a suitable medium such that a PCIP protein is produced. In another embodiment, the method further comprises isolating a PCIP protein from the medium or the host cell.

The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which PCIP-coding sequences have been

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introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous PCIP sequences have been introduced into their genome or homologous recombinant animals in which endogenous PCIP sequences have been altered. Such animals are useful for studying the function and/or activity of a PCIP and for identifying and/or evaluating modulators of PCIP activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous PCIP gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing a PCIP-encoding nucleic acid into the male pronuclei of a fertilized oocyte, *e.g.*, by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The PCIP cDNA sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of a human PCIP gene, such as a mouse or rat PCIP gene, can be used as a transgene. Alternatively, a PCIP gene homologue, such as another PCIP family member, can be isolated based on hybridization to the PCIP cDNA sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID

NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71 or the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994 (described further in subsection I above) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a PCIP transgene to direct expression of a PCIP protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder *et al.*, U.S. Patent No. 4,873,191 by Wagner *et al.* and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of a PCIP transgene in its genome and/or expression of PCIP mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a PCIP protein can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a PCIP gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the PCIP gene. The PCIP gene can be a human gene (*e.g.*, the cDNA of SEQ ID NO:1), but more preferably, is a non-human homologue of a human PCIP gene (*e.g.*, the cDNA of SEQ ID NO:3 or 5). For example, a mouse PCIP gene can be used to construct a homologous recombination vector suitable for altering an endogenous PCIP gene in the mouse genome. In a preferred embodiment, the vector is designed such that, upon homologous

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recombination, the endogenous PCIP gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous PCIP gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous PCIP protein). In the homologous recombination vector, the altered portion of the PCIP gene is flanked at its 5' and 3' ends by additional nucleic acid sequence of the PCIP gene to allow for homologous recombination to occur between the exogenous PCIP gene carried by the vector and an endogenous PCIP gene in an embryonic stem cell. The additional flanking PCIP nucleic acid sequence is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector (see *e.g.*, Thomas, K.R. and Capecchi, M. R. (1987) *Cell* 51:503 for a description of homologous recombination vectors). The vector is introduced into an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced PCIP gene has homologously recombined with the endogenous PCIP gene are selected (see *e.g.*, Li, E. *et al.* (1992) *Cell* 69:915). The selected cells are then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras (see *e.g.*, Bradley, A. in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley, A. (1991) *Current Opinion in Biotechnology* 2:823-829 and in PCT International Publication Nos.: WO 90/11354 by Le Mouellec *et al.*; WO 91/01140 by Smithies *et al.*; WO 92/0968 by Zijlstra *et al.*; and WO 93/04169 by Berns *et al.*

In another embodiment, transgenic non-humans animals can be produced which contain selected systems which allow for regulated expression of the transgene. One example of such a system is the *cre/loxP* recombinase system of bacteriophage P1. For

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a description of the *cre/loxP* recombinase system, see, *e.g.*, Lakso *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al.* (1991) *Science* 251:1351-1355. If a *cre/loxP* recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the *Cre* recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, I. *et al.* (1997) *Nature* 385:810-813 and PCT International Publication Nos. WO 97/07668 and WO 97/07669. In brief, a cell, *e.g.*, a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G₀ phase. The quiescent cell can then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, *e.g.*, the somatic cell, is isolated.

IV. Pharmaceutical Compositions

The PCIP nucleic acid molecules, fragments of PCIP proteins, and anti-PCIP antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof

in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include
5 parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents;
10 antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be
15 enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For
20 intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as
25 bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of
30 surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol,

ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, a fragment of a PCIP protein or an anti-PCIP antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

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For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For
5 transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active
10 compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

15 In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid.
20 Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled
25 in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound
30 calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are

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dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

Toxicity and therapeutic efficacy of such compounds can be determined by
5 standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. While
10 compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies
15 preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a
20 circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

25 As defined herein, a therapeutically effective amount of protein or polypeptide (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The skilled artisan will appreciate that certain
30 factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health

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and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments.

In a preferred example, a subject is treated with antibody, protein, or polypeptide
5 in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody, protein, or polypeptide used for treatment may
10 increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein.

The present invention encompasses agents which modulate expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics, amino acids, amino
15 acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (i.e., including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000
20 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

It is understood that appropriate doses of small molecule agents depends upon a
25 number of factors within the ken of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the small molecule will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the small molecule to have upon the nucleic acid or
30 polypeptide of the invention.

Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (*e.g.*, about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram.

5 It is

furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. Such appropriate doses may be determined using the assays described herein. When one or more of these small molecules is to be administered to an animal (*e.g.*, a human) in

10 order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of
15 the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

Further, an antibody (or fragment thereof) may be conjugated to a therapeutic
20 moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone,
25 glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan,
30 dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and

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doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, alpha.-interferon, beta.-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophase colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, *e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.*, 62:119-58 (1982). Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by

stereotactic injection (see *e.g.*, Chen *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

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V. Uses and Methods of the Invention

The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) screening assays; b) predictive medicine (*e.g.*, diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenetics); and c) methods of treatment (*e.g.*, therapeutic and prophylactic). As described herein, a PCIP protein of the invention has one or more of the following activities: (1) it interacts with (*e.g.*, binds to) a potassium channel protein or portion thereof; (2) it regulates the phosphorylation state of a potassium channel protein or portion thereof; (3) it associates with (*e.g.*, binds to) calcium and can, for example, act as a calcium dependent kinase, *e.g.*, phosphorylate a potassium channel or a G-protein coupled receptor in a calcium-dependent manner; (4) it associates with (*e.g.*, binds to) calcium and can, for example, act as a calcium dependent transcription factor; (5) it modulates a potassium channel mediated activity in a cell (*e.g.*, a neuronal or cardiac cell) to, for example, beneficially affect the cell; (6) it modulates chromatin formation in a cell, *e.g.*, a neuronal or cardiac cell; (7) it modulates vesicular traffic and protein transport in a cell, *e.g.*, a neuronal or cardiac cell; (8) it modulates cytokine signaling in a cell, *e.g.*, a neuronal or cardiac cell; (9) it regulates the association of a potassium channel protein or portion thereof with the cellular cytoskeleton; (10) it modulates cellular proliferation; (11) it modulates the release of neurotransmitters; (12) it modulates membrane excitability; (13) it influences the resting potential of membranes; (14) it modulates wave forms and frequencies of action potentials; and (15)

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it modulates thresholds of excitation and, thus, can be used to, for example, (1) modulate the activity of a potassium channel protein or portion thereof; (2) modulate the phosphorylation state of a potassium channel protein or portion thereof; (3) modulate the phosphorylation state of a potassium channel or a G-protein coupled receptor in a calcium-dependent manner; (4) associate with (*e.g.*, bind to) calcium and act as a calcium dependent transcription factor; (5) modulate a potassium channel mediated activity in a cell (*e.g.*, a neuronal or cardiac cell) to, for example, beneficially affect the cell; (6) modulate chromatin formation in a cell, *e.g.*, a neuronal or cardiac cell; (7) modulate vesicular traffic and protein transport in a cell, *e.g.*, a neuronal or cardiac cell; (8) modulate cytokine signaling in a cell, *e.g.*, a neuronal or cardiac cell; (9) regulate the association of a potassium channel protein or portion thereof with the cellular cytoskeleton; (10) modulate cellular proliferation; (11) modulate the release of neurotransmitters; (12) modulate membrane excitability; (13) influence the resting potential of membranes; (14) modulate wave forms and frequencies of action potentials; and (15) modulate thresholds of excitation.

The isolated nucleic acid molecules of the invention can be used, for example, to express PCIP protein (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications), to detect PCIP mRNA (*e.g.*, in a biological sample) or a genetic alteration in a PCIP gene, and to modulate PCIP activity, as described further below.

The PCIP proteins can be used to treat disorders characterized by insufficient or excessive production of a PCIP substrate or production of PCIP inhibitors. In addition, the PCIP proteins can be used to screen for naturally occurring PCIP substrates, to screen for drugs or compounds which modulate PCIP activity, as well as to treat disorders characterized by insufficient or excessive production of PCIP protein or production of PCIP protein forms which have decreased or aberrant activity compared to PCIP wild type protein (*e.g.*, CNS disorders such as neurodegenerative disorders, *e.g.*, Alzheimer's disease, dementias related to Alzheimer's disease (such as Pick's disease), Parkinson's and other Lewy diffuse body diseases, multiple sclerosis, amyotrophic lateral sclerosis, progressive supranuclear palsy, epilepsy, spinocerebellar ataxia, and Jakob-Creutzfeldt disease; psychiatric disorders, *e.g.*, depression, schizophrenic disorders, Korsakoff's psychosis, mania, anxiety disorders, bipolar affective disorders, or

phobic disorders; learning or memory disorders, *e.g.*, amnesia or age-related memory loss; neurological disorders, *e.g.*, migraine; pain disorders, *e.g.*, hyperalgesia or pain associated with musculoskeletal disorders; spinal cord injury; stroke; and head trauma; or cardiovascular disorders such as sinus node dysfunction, angina, heart failure,

- 5 hypertension, atrial fibrillation, atrial flutter, dilated cardiomyopathy, idiopathic cardiomyopathy, myocardial infarction, coronary artery disease, coronary artery spasm, or arrhythmia). Moreover, the anti-PCIP antibodies of the invention can be used to detect and isolate PCIP proteins, regulate the bioavailability of PCIP proteins, and modulate PCIP activity.

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A. Screening Assays:

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) which bind to PCIP proteins, have a
15 stimulatory or inhibitory effect on, for example, PCIP expression or PCIP activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of PCIP substrate.

In one embodiment, the invention provides assays for screening candidate or test compounds which are substrates of a PCIP protein or polypeptide or biologically active
20 portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a PCIP protein or polypeptide or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable
25 parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997)
30 *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994). *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP 10 '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra.*).

In one embodiment, an assay is a cell-based assay in which a cell which 15 expresses a PCIP protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate PCIP activity, *e.g.*, binding to a potassium channel or a portion thereof, is determined. Determining the ability of the test compound to modulate PCIP activity can be accomplished by monitoring, for example, the release of a neurotransmitter, *e.g.*, dopamine, from a cell which expresses 20 PCIP such as a neuronal cell, *e.g.*, a substantia nigra neuronal cell, or a cardiac cell. Furthermore, determining the ability of the test compound to modulate PCIP activity can be accomplished by monitoring, for example, the I_{to} current or the release of a neurotransmitter from a cell which expresses PCIP such as a cardiac cell. Currents in cells, *e.g.*, the I_{to} current, can be measured using the patch-clamp technique as described 25 in the Examples section using the techniques described in, for example, Hamill *et al.* 1981. *Pfluegers Arch.* 391: 85-100). The cell, for example, can be of mammalian origin. Determining the ability of the test compound to modulate the ability of PCIP to bind to a substrate can be accomplished, for example, by coupling the PCIP substrate with a radioisotope or enzymatic label such that binding of the PCIP substrate to PCIP can be 30 determined by detecting the labeled PCIP substrate in a complex. For example, compounds (*e.g.*, PCIP substrates) can be labeled with ^{125}I , ^{35}S , ^{14}C , or ^3H , either

directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

It is also within the scope of this invention to determine the ability of a compound (*e.g.*, PCIP substrate) to interact with PCIP without the labeling of any of the interactants. For example, a microphysiometer can be used to detect the interaction of a compound with PCIP without the labeling of either the compound or the PCIP.

McConnell, H. M. *et al.* (1992) *Science* 257:1906-1912. As used herein, a "microphysiometer" (*e.g.*, Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and PCIP.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a PCIP target molecule (*e.g.*, a potassium channel or a fragment thereof) with a test compound and determining the ability of the test compound to modulate (*e.g.* stimulate or inhibit) the activity of the PCIP target molecule. Determining the ability of the test compound to modulate the activity of a PCIP target molecule can be accomplished, for example, by determining the ability of the PCIP protein to bind to or interact with the PCIP target molecule, *e.g.*, a potassium channel or a fragment thereof.

Determining the ability of the PCIP protein or a biologically active fragment thereof, to bind to or interact with a PCIP target molecule can be accomplished by one of the methods described above for determining direct binding. In a preferred embodiment, determining the ability of the PCIP protein to bind to or interact with a PCIP target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.*, intracellular Ca^{2+} , diacylglycerol, IP_3 , and the like), detecting catalytic/enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising a target-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*,

luciferase), or detecting a target-regulated cellular response such as the release of a neurotransmitter.

In yet another embodiment, an assay of the present invention is a cell-free assay in which a PCIP protein or biologically active portion thereof is contacted with a test
5 compound and the ability of the test compound to bind to the PCIP protein or biologically active portion thereof is determined. Preferred biologically active portions of the PCIP proteins to be used in assays of the present invention include fragments which participate in interactions with non-PCIP molecules, *e.g.*, potassium channels or fragments thereof, or fragments with high surface probability scores. Binding of the test
10 compound to the PCIP protein can be determined either directly or indirectly as described above. In a preferred embodiment, the assay includes contacting the PCIP protein or biologically active portion thereof with a known compound which binds PCIP to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a PCIP protein, wherein
15 determining the ability of the test compound to interact with a PCIP protein comprises determining the ability of the test compound to preferentially bind to PCIP or biologically active portion thereof as compared to the known compound.

In another embodiment, the assay is a cell-free assay in which a PCIP protein or biologically active portion thereof is contacted with a test compound and the ability of
20 the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the PCIP protein or biologically active portion thereof is determined. Determining the ability of the test compound to modulate the activity of a PCIP protein can be accomplished, for example, by determining the ability of the PCIP protein to bind to a PCIP target molecule by one of the methods described above for determining direct binding. Determining the ability
25 of the PCIP protein to bind to a PCIP target molecule can also be accomplished using a technology such as real-time Biomolecular Interaction Analysis (BIA). Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705. As used herein, "BIA" is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*,
30 BIAcore). Changes in the optical phenomenon of surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In an alternative embodiment, determining the ability of the test compound to modulate the activity of a PCIP protein can be accomplished by determining the ability of the PCIP protein to further modulate the activity of a downstream effector of a PCIP target molecule. For example, the activity of the effector molecule on an appropriate target can be determined or the binding of the effector to an appropriate target can be determined as previously described.

In yet another embodiment, the cell-free assay involves contacting a PCIP protein or biologically active portion thereof with a known compound which binds the PCIP protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the PCIP protein, wherein determining the ability of the test compound to interact with the PCIP protein comprises determining the ability of the PCIP protein to preferentially bind to or modulate the activity of a PCIP target molecule.

The cell-free assays of the present invention are amenable to use of both soluble and/or membrane-bound forms of isolated proteins. In the case of cell-free assays in which a membrane-bound form of an isolated protein is used (*e.g.*, a potassium channel) it may be desirable to utilize a solubilizing agent such that the membrane-bound form of the isolated protein is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton[®] X-100, Triton[®] X-114, Thesit[®], Isotridecypoly(ethylene glycol ether)_n, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl=N,N-dimethyl-3-ammonio-1-propane sulfonate.

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either PCIP or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a PCIP protein, or interaction of a PCIP protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates,

test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/ PCIP fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads

5 (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtitre plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or PCIP protein, and the mixture incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtitre plate wells are washed to remove any unbound

10 components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of PCIP binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the

15 screening assays of the invention. For example, either a PCIP protein or a PCIP target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated PCIP protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well

20 plates (Pierce Chemical). Alternatively, antibodies reactive with PCIP protein or target molecules but which do not interfere with binding of the PCIP protein to its target molecule can be derivatized to the wells of the plate, and unbound target or PCIP protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include

25 immunodetection of complexes using antibodies reactive with the PCIP protein or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the PCIP protein or target molecule.

In a preferred embodiment, candidate or test compounds or agents are tested for their ability to inhibit or stimulate a PCIP molecule's ability to modulate vesicular traffic

30 and protein transport in a cell, *e.g.*, a neuronal or cardiac cell, using the assays described in, for example, Komada M. *et al.* (1999) *Genes Dev.*13(11):1475-85, and Roth M.G. *et*

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al. (1999) *Chem. Phys. Lipids*. 98(1-2):141-52, the contents of which are incorporated herein by reference.

In another preferred embodiment, candidate or test compounds or agents are tested for their ability to inhibit or stimulate a PCIP molecule's ability to regulate the phosphorylation state of a potassium channel protein or portion thereof, using for example, an *in vitro* kinase assay. Briefly, a PCIP target molecule, *e.g.*, an immunoprecipitated potassium channel from a cell line expressing such a molecule, can be incubated with the PCIP protein and radioactive ATP, *e.g.*, [γ - ^{32}P] ATP, in a buffer containing MgCl_2 and MnCl_2 , *e.g.*, 10 mM MgCl_2 and 5 mM MnCl_2 . Following the incubation, the immunoprecipitated PCIP target molecule, *e.g.*, the potassium channel, can be separated by SDS-polyacrylamide gel electrophoresis under reducing conditions, transferred to a membrane, *e.g.*, a PVDF membrane, and autoradiographed. The appearance of detectable bands on the autoradiograph indicates that the PCIP substrate, *e.g.*, the potassium channel, has been phosphorylated. Phosphoaminoacid analysis of the phosphorylated substrate can also be performed in order to determine which residues on the PCIP substrate are phosphorylated. Briefly, the radiophosphorylated protein band can be excised from the SDS gel and subjected to partial acid hydrolysis. The products can then be separated by one-dimensional electrophoresis and analyzed on, for example, a phosphoimager and compared to ninhydrin-stained phosphoaminoacid standards. Assays such as those described in, for example, Tamaskovic R. *et al.* (1999) *Biol. Chem.* 380(5):569-78, the contents of which are incorporated herein by reference, can also be used.

In another preferred embodiment, candidate or test compounds or agents are tested for their ability to inhibit or stimulate a PCIP molecule's ability to associate with (*e.g.*, bind) calcium, using for example, the assays described in Liu L. (1999) *Cell Signal*. 11(5):317-24 and Kawai T. *et al.* (1999) *Oncogene* 18(23):3471-80, the contents of which are incorporated herein by reference.

In another preferred embodiment, candidate or test compounds or agents are tested for their ability to inhibit or stimulate a PCIP molecule's ability to modulate chromatin formation in a cell, using for example, the assays described in Okuwaki M. *et*

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al. (1998) *J. Biol. Chem.* 273(51):34511-8 and Miyaji-Yamaguchi M. (1999) *J. Mol. Biol.* 290(2): 547-557, the contents of which are incorporated herein by reference.

In yet another preferred embodiment, candidate or test compounds or agents are tested for their ability to inhibit or stimulate a PCIP molecule's ability to modulate cellular proliferation, using for example, the assays described in Baker F.L. *et al.* (1995) *Cell Prolif.* 28(1):1-15, Cheviron N. *et al.* (1996) *Cell Prolif.* 29(8):437-46, Hu Z.W. *et al.* (1999) *J. Pharmacol. Exp. Ther.* 290(1):28-37 and Elliott K. *et al.* (1999) *Oncogene* 18(24):3564-73, the contents of which are incorporated herein by reference.

In a preferred embodiment, candidate or test compounds or agents are tested for their ability to inhibit or stimulate a PCIP molecule's ability to regulate the association of a potassium channel protein or portion thereof with the cellular cytoskeleton, using for example, the assays described in Gonzalez C. *et al.* (1998) *Cell Mol. Biol.* 44(7):1117-27 and Chia C.P. *et al.* (1998) *Exp. Cell Res.* 244(1):340-8, the contents of which are incorporated herein by reference.

In another preferred embodiment, candidate or test compounds or agents are tested for their ability to inhibit or stimulate a PCIP molecule's ability to modulate membrane excitability, using for example, the assays described in Bar-Sagi D. *et al.* (1985) *J. Biol. Chem.* 260(8):4740-4 and Barker J.L. *et al.* (1984) *Neurosci. Lett.* 47(3):313-8, the contents of which are incorporated herein by reference.

In another preferred embodiment, candidate or test compounds or agents are tested for their ability to inhibit or stimulate a PCIP molecule's ability to modulate cytokine signaling in a cell, *e.g.*, a neuronal or cardiac cell, the assays described in Nakashima Y. *et al.* (1999) *J. Bone Joint Surg. Am.* 81(5):603-15, the contents of which are incorporated herein by reference.

In another embodiment, modulators of PCIP expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of PCIP mRNA or protein in the cell is determined. The level of expression of PCIP mRNA or protein in the presence of the candidate compound is compared to the level of expression of PCIP mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of PCIP expression based on this comparison. For example, when expression of PCIP mRNA or protein is greater

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(statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of PCIP mRNA or protein expression. Alternatively, when expression of PCIP mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of PCIP mRNA or protein expression. The level of PCIP mRNA or protein expression in the cells can be determined by methods described herein for detecting PCIP mRNA or protein.

In yet another aspect of the invention, the PCIP proteins can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.* 268:12046-12054; Bartel *et al.* (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with PCIP ("PCIP-binding proteins" or "PCIP-bp") and are involved in PCIP activity (described in more detail in the Examples section below).

Such PCIP-binding proteins are also likely to be involved in the propagation of signals by the PCIP proteins or PCIP targets as, for example, downstream elements of a PCIP-mediated signaling pathway. Alternatively, such PCIP-binding proteins are likely to be PCIP inhibitors.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a PCIP protein is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming a PCIP-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies

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containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the PCIP protein.

This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein in an appropriate animal model. For example, an agent identified as described herein (*e.g.*, a PCIP modulating agent, an antisense PCIP nucleic acid molecule, a PCIP-specific antibody, or a PCIP-binding partner) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments, *e.g.*, treatments of a CNS disorder or a cardiovascular disorder, as described herein.

B. Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

1. Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the PCIP nucleotide sequences, described herein, can be used to map the location of the PCIP genes on a chromosome. The mapping of the PCIP sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, PCIP genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the PCIP nucleotide sequences. Computer analysis of the PCIP sequences can be used to predict primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers
 5 can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the PCIP sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (*e.g.*, human and mouse cells). As hybrids of human and mouse cells grow
 10 and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but human cells can, the one human chromosome that contains the gene encoding the needed enzyme, will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single
 15 human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. (D'Eustachio P. *et al.* (1983) *Science* 220:919-924). Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

20 PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the PCIP nucleotide sequences to design oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes. Other mapping strategies which can similarly be
 25 used to map a PCIP sequence to its chromosome include *in situ* hybridization (described in Fan, Y. *et al.* (1990) *Proc. Natl. Acad. Sci. USA*, 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase
 30 chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been

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blocked in metaphase by a chemical such as colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence
 5 as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases will suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma *et al.*, Human Chromosomes: A Manual of Basic Techniques
 10 (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding
 15 sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in
 20 Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland, J. *et al.* (1987) *Nature*, 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and
 25 unaffected with a disease associated with the PCIP gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are
 30 visible from chromosome spreads or detectable using PCR based on that DNA sequence.

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Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

2. Tissue Typing

5 The PCIP sequences of the present invention can also be used to identify individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield
10 unique bands for identification. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

 Furthermore, the sequences of the present invention can be used to provide an
15 alternative technique which determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the PCIP nucleotide sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

20 Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from individuals and from tissue. The PCIP nucleotide sequences of the invention uniquely represent
25 portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared
30 for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. Non-

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coding sequences can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

5 If a panel of reagents from PCIP nucleotide sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from extremely small tissue samples.

10

3. Use of Partial PCIP Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology.

Forensic biology is a scientific field employing genetic typing of biological evidence found at a crime scene as a means for positively identifying, for example, a perpetrator
15 of a crime. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, *e.g.*, hair or skin, or body fluids, *e.g.*, blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

20 The sequences of the present invention can be used to provide polynucleotide reagents, *e.g.*, PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (*i.e.* another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for
25 identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions are particularly appropriate for this use as greater numbers of polymorphisms occur in the noncoding regions, making it easier to differentiate individuals using this technique. Examples of polynucleotide reagents include the PCIP nucleotide sequences or portions thereof,
30 having a length of at least 20 bases, preferably at least 30 bases.

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The PCIP nucleotide sequences described herein can further be used to provide polynucleotide reagents, *e.g.*, labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue, *e.g.*, brain tissue. This can be very useful in cases where a forensic pathologist is presented with a
5 tissue of unknown origin. Panels of such PCIP probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, *e.g.*, PCIP primers or probes can be used to screen tissue culture for contamination (*i.e.* screen for the presence of a mixture of different types of cells in a culture).

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C. Predictive Medicine:

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically.
15 Accordingly, one aspect of the present invention relates to diagnostic assays for determining PCIP protein and/or nucleic acid expression as well as PCIP activity, in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant PCIP expression or activity. The invention also
20 provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with PCIP protein, nucleic acid expression or activity. For example, mutations in a PCIP gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with
25 PCIP protein, nucleic acid expression or activity.

Another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of PCIP in clinical trials.

These and other agents are described in further detail in the following sections.

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1. Diagnostic Assays

An exemplary method for detecting the presence or absence of PCIP protein or nucleic acid in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting PCIP protein or nucleic acid (*e.g.*, mRNA, genomic DNA) that encodes PCIP protein such that the presence of PCIP protein or nucleic acid is detected in the biological sample. A preferred agent for detecting PCIP mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to PCIP mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length PCIP nucleic acid, such as the nucleic acid of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, or 98994, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to PCIP mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

A preferred agent for detecting PCIP protein is an antibody capable of binding to PCIP protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or F(ab')₂) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with

biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect PCIP mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of PCIP mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of PCIP protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of PCIP genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of PCIP protein include introducing into a subject a labeled anti-PCIP antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a serum sample or cerebrospinal fluid isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting PCIP protein, mRNA, or genomic DNA, such that the presence of PCIP protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of PCIP protein, mRNA or genomic DNA in the control sample with the presence of PCIP protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of PCIP in a biological sample. For example, the kit can comprise a labeled compound or agent capable of detecting PCIP protein or mRNA in a biological sample; means for determining the amount of PCIP in the sample; and means for comparing the amount of PCIP in the sample with a standard. The compound or agent can be packaged in a

suitable container. The kit can further comprise instructions for using the kit to detect PCIP protein or nucleic acid.

2. Prognostic Assays

- 5 The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant PCIP expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with a misregulation in PCIP
- 10 protein activity or nucleic acid expression, such as a neurodegenerative disorder, *e.g.*, Alzheimer's disease, dementias related to Alzheimer's disease (such as Pick's disease), Parkinson's and other Lewy diffuse body diseases, multiple sclerosis, amyotrophic lateral sclerosis, progressive supranuclear palsy, epilepsy, spinocerebellar ataxia, and Jakob-Creutzfeldt disease; a psychiatric disorder, *e.g.*, depression, schizophrenic
- 15 disorders, Korsakoff's psychosis, mania, anxiety disorders, bipolar affective disorders, or phobic disorders; a learning or memory disorder, *e.g.*, amnesia or age-related memory loss; a neurological disorder, *e.g.*, migraine; a pain disorder, *e.g.*, hyperalgesia or pain associated with musculoskeletal disorders; spinal cord injury; stroke; and head trauma; or a cardiovascular disorder, *e.g.*, sinus node dysfunction, angina, heart failure,
- 20 hypertension, atrial fibrillation, atrial flutter, dilated cardiomyopathy, idiopathic cardiomyopathy, myocardial infarction, coronary artery disease, coronary artery spasm, or arrhythmia.

Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disorder associated with a misregulation in PCIP protein activity

25 or nucleic acid expression, such as a potassium channel associated disorder. Thus, the present invention provides a method for identifying a disease or disorder associated with aberrant PCIP expression or activity in which a test sample is obtained from a subject and PCIP protein or nucleic acid (*e.g.*, mRNA or genomic DNA) is detected, wherein the presence of PCIP protein or nucleic acid is diagnostic for a subject having or at risk

30 of developing a disease or disorder associated with aberrant PCIP expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of

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interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant PCIP expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a CNS disorder or a cardiovascular disorder. Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant PCIP expression or activity in which a test sample is obtained and PCIP protein or nucleic acid expression or activity is detected (*e.g.*, wherein the abundance of PCIP protein or nucleic acid expression or activity is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant PCIP expression or activity).

The methods of the invention can also be used to detect genetic alterations in a PCIP gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by misregulation in PCIP protein activity or nucleic acid expression, such as a CNS disorder or a cardiovascular disorder. In preferred embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding a PCIP-protein, or the mis-expression of the PCIP gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from a PCIP gene; 2) an addition of one or more nucleotides to a PCIP gene; 3) a substitution of one or more nucleotides of a PCIP gene, 4) a chromosomal rearrangement of a PCIP gene; 5) an alteration in the level of a messenger RNA transcript of a PCIP gene, 6) aberrant modification of a PCIP gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a PCIP gene, 8) a non-wild type level of a PCIP-protein, 9) allelic loss of a PCIP gene, and 10) inappropriate post-translational modification of a PCIP-protein. As described herein, there are a large number of assays known in the art which can be used for detecting alterations in a PCIP

gene. A preferred biological sample is a tissue or serum sample isolated by conventional means from a subject.

In certain embodiments, detection of the alteration involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, *e.g.*, U.S. Patent Nos. 5 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, *e.g.*, Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:360-364), the latter of which can be particularly useful for detecting point mutations in the PCIP-gene (see Abravaya *et al.* (1995) *Nucleic Acids Res.* 23:675-682). This method can include the steps of
10 collecting a sample of cells from a subject, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a PCIP gene under conditions such that hybridization and amplification of the PCIP-gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the
15 amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. *et al.*, (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional
20 amplification system (Kwoh, D.Y. *et al.*, (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. *et al.* (1988) *Bio-Technology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such
25 molecules are present in very low numbers.

In an alternative embodiment, mutations in a PCIP gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel
30 electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence

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specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in PCIP can be identified by

- 5 hybridizing a sample and control nucleic acids, e.g., DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotide probes (Cronin, M.T. *et al.* (1996) *Human Mutation* 7: 244-255; Kozal, M.J. *et al.* (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in PCIP can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. *et al. supra*.
- 10 Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe
- 15 arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the PCIP gene and detect mutations by

- comparing the sequence of the sample PCIP with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxam and Gilbert ((1977) *Proc. Natl. Acad. Sci. USA* 74:560) or Sanger ((1977) *Proc. Natl. Acad. Sci. USA* 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Biotechniques* 19:448), including sequencing by mass spectrometry (see, *e.g.*, PCT International Publication No. WO 94/16101; Cohen *et al.* (1996) *Adv. Chromatogr.* 36:127-162; and Griffin *et al.* (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

Other methods for detecting mutations in the PCIP gene include methods in

- 30 which protection from cleavage agents is used to detect mismatched bases in RNA/RNA
or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the

art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type PCIP sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al.* (1988) *Proc. Natl Acad Sci USA* 85:4397; Saleeba *et al.* (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in PCIP cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on a PCIP sequence, *e.g.*, a wild-type PCIP sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in PCIP genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA*: 86:2766, see also Cotton (1993) *Mutat. Res.* 285:125-144; and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and

control PCIP nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

10 In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl Acad. Sci USA* 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3'

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end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain

5 embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

10 The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a PCIP gene.

15 Furthermore, any cell type or tissue in which PCIP is expressed may be utilized in the prognostic assays described herein.

3. Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (*e.g.*, drugs) on the expression or activity of a

20 PCIP protein (*e.g.*, the modulation of membrane excitability or resting potential) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase PCIP gene expression, protein levels, or upregulate PCIP activity, can be monitored in clinical trials of subjects exhibiting decreased PCIP gene expression, protein levels, or

25 downregulated PCIP activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease PCIP gene expression, protein levels, or downregulate PCIP activity, can be monitored in clinical trials of subjects exhibiting increased PCIP gene expression, protein levels, or upregulated PCIP activity. In such clinical trials, the expression or activity of a PCIP gene, and preferably, other genes that have been

30 implicated in, for example, a potassium channel associated disorder can be used as a "read out" or markers of the phenotype of a particular cell.

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For example, and not by way of limitation, genes, including PCIP, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) which modulates PCIP activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on potassium channel associated disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of PCIP and other genes implicated in the potassium channel associated disorder, respectively. The levels of gene expression (*e.g.*, a gene expression pattern) can be quantified by northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of PCIP or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during treatment of the individual with the agent.

In a preferred embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) including the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a PCIP protein, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the PCIP protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the PCIP protein, mRNA, or genomic DNA in the pre-administration sample with the PCIP protein, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of PCIP to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of PCIP to lower levels than detected, *i.e.* to decrease the effectiveness of the agent. According to such an

embodiment, PCIP expression or activity may be used as an indicator of the effectiveness of an agent, even in the absence of an observable phenotypic response.

D. Methods of Treatment:

- 5 The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant PCIP expression or activity. With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics.
- 10 "Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers the study of how a patient's genes determine his or her response to a drug (*e.g.*, a patient's "drug response phenotype", or "drug response genotype".) Thus, another aspect of the
- 15 invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the PCIP molecules of the present invention or PCIP modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will
- 20 experience toxic drug-related side effects.

1. Prophylactic Methods

- In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant PCIP expression or activity, by
- 25 administering to the subject a PCIP or an agent which modulates PCIP expression or at least one PCIP activity. Subjects at risk for a disease which is caused or contributed to by aberrant PCIP expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the
- 30 PCIP aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of PCIP aberrancy, for example, a PCIP, PCIP

agonist or PCIP antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

2. Therapeutic Methods

5 Another aspect of the invention pertains to methods of modulating PCIP expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell with a PCIP or agent that modulates one or more of the activities of PCIP protein activity associated with the cell. An agent that modulates PCIP protein activity can be an agent
10 as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of a PCIP protein (*e.g.*, a PCIP substrate), a PCIP antibody, a PCIP agonist or antagonist, a peptidomimetic of a PCIP agonist or antagonist, or other small molecule. In one embodiment, the agent stimulates one or more PCIP activities. Examples of such stimulatory agents include active PCIP protein and a nucleic acid molecule encoding
15 PCIP that has been introduced into the cell. In another embodiment, the agent inhibits one or more PCIP activities. Examples of such inhibitory agents include antisense PCIP nucleic acid molecules, anti-PCIP antibodies, and PCIP inhibitors. These modulatory methods can be performed *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject). As such, the present
20 invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of a PCIP protein or nucleic acid molecule. Examples of such disorders include CNS disorders such as neurodegenerative disorders, *e.g.*, Alzheimer's disease, dementias related to Alzheimer's disease (such as Pick's disease), Parkinson's and other Lewy diffuse body diseases, multiple sclerosis, amyotrophic lateral sclerosis, progressive supranuclear palsy, epilepsy, and Jakob-
25 Creutzfeldt disease; psychiatric disorders, *e.g.*, depression, schizophrenic disorders, Korsakoff's psychosis, mania, anxiety disorders, bipolar affective disorders, or phobic disorders; learning or memory disorders, *e.g.*, amnesia or age-related memory loss; neurological disorders, *e.g.*, migraine; pain disorders, *e.g.*, hyperalgesia or pain
30 associated with musculoskeletal disorders; spinal cord injury; stroke; and head trauma; or cardiovascular disorders, *e.g.*, arteriosclerosis, ischemia reperfusion injury, restenosis,

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- arterial inflammation, vascular wall remodeling, ventricular remodeling, rapid ventricular pacing, coronary microembolism, tachycardia, bradycardia, pressure overload, aortic bending, coronary artery ligation, vascular heart disease, atrial fibrillation, long-QT syndrome, congestive heart failure, sinus node dysfunction, angina,
- 5 heart failure, hypertension, atrial fibrillation, atrial flutter, dilated cardiomyopathy, idiopathic cardiomyopathy, myocardial infarction, coronary artery disease, coronary artery spasm, or arrhythmia. In one embodiment, the method involves administering an agent (*e.g.*, an agent identified by a screening assay described herein), or combination of agents that modulates (*e.g.*, upregulates or downregulates) PCIP expression or activity.
- 10 In another embodiment, the method involves administering a PCIP protein or nucleic acid molecule as therapy to compensate for reduced or aberrant PCIP expression or activity.

- A preferred embodiment of the present invention involves a method for treatment of a PCIP associated disease or disorder which includes the step of administering a
- 15 therapeutically effective amount of a PCIP antibody to a subject. As defined herein, a therapeutically effective amount of antibody (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The
- 20 skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of an antibody can include a single treatment or, preferably, can include a series
- 25 of treatments. In a preferred example, a subject is treated with antibody in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody used for treatment may increase or decrease over
- 30 the course of a particular treatment. Changes in dosage may result from the results of diagnostic assays as described herein.

Stimulation of PCIP activity is desirable in situations in which PCIP is abnormally downregulated and/or in which increased PCIP activity is likely to have a beneficial effect. For example, stimulation of PCIP activity is desirable in situations in which a PCIP is downregulated and/or in which increased PCIP activity is likely to have a beneficial effect. Likewise, inhibition of PCIP activity is desirable in situations in which PCIP is abnormally upregulated and/or in which decreased PCIP activity is likely to have a beneficial effect.

3. Pharmacogenomics

The PCIP molecules of the present invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on PCIP activity (*e.g.*, PCIP gene expression) as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) potassium channel associated disorders associated with aberrant PCIP activity (*e.g.*, CNS disorders such as neurodegenerative disorders, *e.g.*, Alzheimer's disease, dementias related to Alzheimer's disease (such as Pick's disease), Parkinson's and other Lewy diffuse body diseases, multiple sclerosis, amyotrophic lateral sclerosis, progressive supranuclear palsy, epilepsy, spinocerebellar ataxia, and Jakob-Creutzfeldt disease; psychiatric disorders, *e.g.*, depression, schizophrenic disorders, Korsakoff's psychosis, mania, anxiety disorders, bipolar affective disorders, or phobic disorders; learning or memory disorders, *e.g.*, amnesia or age-related memory loss; neurological disorders, *e.g.*, migraine; pain disorders, *e.g.*, hyperalgesia or pain associated with musculoskeletal disorders; spinal cord injury; stroke; and head trauma; or cardiovascular disorders such as arteriosclerosis, ischemia reperfusion injury, restenosis, arterial inflammation, vascular wall remodeling, ventricular remodeling, rapid ventricular pacing, coronary microembolism, tachycardia, bradycardia, pressure overload, aortic bending, coronary artery ligation, vascular heart disease, atrial fibrillation, long-QT syndrome, congestive heart failure, sinus node dysfunction, angina, heart failure, hypertension, atrial fibrillation, atrial flutter, dilated cardiomyopathy, idiopathic cardiomyopathy, myocardial infarction, coronary artery disease, coronary artery spasm, or arrhythmia). In conjunction with such treatment, pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype

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and that individual's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer a PCIP molecule or PCIP modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with a PCIP molecule or PCIP modulator.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum, M. *et al.* (1996) *Clin. Exp. Pharmacol. Physiol.* 23(10-11) :983-985 and Linder, M.W. *et al.* (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (*e.g.*, a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of

DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drugs target is known (*e.g.*, a PCIP protein of the present invention), all common variants of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

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Alternatively, a method termed the "gene expression profiling", can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (*e.g.*, a PCIP molecule or PCIP modulator of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a PCIP molecule or PCIP modulator, such as a modulator identified by one of the exemplary screening assays described herein.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the Figures and the Sequence Listing are incorporated herein by reference.

EXAMPLES

The following materials and methods were used in the Examples.

Strains, plasmids, bait cDNAs, and general microbiological techniques

Basic yeast strains (HF7c, Y187,) bait (pGBT9) and fish (pACT2) plasmids used in this work were purchased from Clontech (Palo Alto, CA). cDNAs encoding rat Kv4.3, Kv4.2, and Kv1.1, were provided by Wyeth-Ayerst Research (865 Ridge Rd., Monmouth Junction, NJ 08852) Standard yeast media including synthetic complete medium lacking L-leucine, L-tryptophan, and L-histidine were prepared and yeast genetic manipulations were performed as described (Sherman (1991) *Meth. Enzymol.* 194:3-21). Yeast transformations were performed using standard protocols (Gietz *et al.* (1992) *Nucleic Acids Res.* 20:1425; Ito *et al* (1983) *J. Bacteriol.* 153:163-168). Plasmid

DNAs were isolated from yeast strains by a standard method (Hoffman and Winston (1987) *Gene* 57:267-272).

Bait and Yeast Strain Construction

5 The first 180 amino acids of rKv4.3 (described in Serdio P. *et al.* (1996) *J. Neurophys* 75:2174-2179) were amplified by PCR and cloned in frame into pGBT9 resulting in plasmid pFWA2, (hereinafter "bait"). This bait was transformed into the two-hybrid screening strain HF7c and tested for expression and self-activation. The bait was validated for expression by Western blotting. The rKv4.3 bait did not self-activate
10 in the presence of 10 mM 3-amino-1,2,3-Triazole (3-AT).

Library construction

 Rat mid brain tissue was provided by Wyeth-Ayerst Research (Monmouth Junction, NJ). Total cellular RNA was extracted from the tissues using standard
15 techniques (Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, (1989)). mRNA was prepared using a Poly-A Spin mRNA Isolation Kit from New England Biolabs (Beverly, MA). cDNA from the mRNA sample was synthesized using a cDNA Synthesis Kit from Stratagene (La
20 Jolla, CA) and ligated into pACT2's EcoRI and XhoI sites, giving rise to a two-hybrid library.

Two-Hybrid Screening

 Two-hybrid screens were carried out essentially as described in Bartel, P. *et al.*
25 (1993) "Using the Two-Hybrid System to Detect Polypeptide-Polypeptide Interactions" in *Cellular Interactions in Development: A Practical Approach*, Hartley, D.A. ed. Oxford University Press, Oxford, pp. 153-179, with a bait-library pair of rkv4.3 bait-rat mid brain library. A filter disk beta-galactosidase (beta-gal) assay was performed essentially as previously described (Brill *et al.* (1994) *Mol. Biol. Cell.* 5:297-312). Clones that were
30 positive for both reporter gene activity (His and beta-galactosidase) were scored and fish, plasmids were isolated from yeast, transformed into *E. coli* strain KC8, DNA

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plasmids were purified and the resulting plasmids were sequenced by conventional methods (Sanger F. *et al.* (1977) *PNAS*, 74: 5463-67).

Specificity test

5 Positive interactor clones were subjected to a binding specificity test where they were exposed to a panel of related and unrelated baits by a mating scheme previously described (Finley R.L. Jr. *et al.* (1994) *PNAS*, 91(26):12980-12984). Briefly, positive fish plasmids were transformed into Y187 and the panel of baits were transformed into HF7c. Transformed fish and bait cells were streaked out as stripes on selective medium
10 plates, mated on YPAD plates, and tested for reporter gene activity.

Analysis

PCIP nucleotides were analyzed for nucleic acid hits by the BLASTN 1.4.8MP program (Altschul *et al.* (1990) Basic Local Alignment Search Tool. *J. Mol. Biol.* 215:
15 403-410). PCIP proteins were analyzed for polypeptide hits by the BLASTP 1.4.9MP program.

EXAMPLE 1: IDENTIFICATION OF RAT PCIP cDNAs

The Kv4.3 gene coding sequence (coding for the first 180 amino acids) was
20 amplified by PCR and cloned into pGBT9 creating a GAL4 DNA-binding domain-Kv4.3(1-180) gene fusion (plasmid pFWA2). HF7c was transformed with this construct. The resulting strain grew on synthetic complete medium lacking L-tryptophan but not on synthetic complete medium lacking L-tryptophan and L-histidine in the presence of 10mM 3-AT demonstrating that the {GAL4 DNA-binding domain}-
25 {vKv4.3(1-180)} gene fusion does not have intrinsic transcriptional activation activity higher than the threshold allowed by 10mM 3-AT .

In this example, a yeast two-hybrid assay was performed in which a plasmid containing a {GAL4 DNA-binding domain}-{rKv4.3(1-180)} gene fusion was introduced into the yeast two-hybrid screening strain HF7c described above. HF7c was
30 then transformed with the rat mid brain two-hybrid library. Approximately six million transformants were obtained and plated in selection medium. Colonies that grew in the

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selection medium and expressed the beta-galactosidase reporter gene were further characterized and subjected to retransformation and specificity assays. The retransformation and specificity tests yielded three PCIP clones (rat 1v, 8t, and 9qm) that were able to bind to the Kv4.3 polypeptide.

5 The full length sequences for the rat 1v gene, and partial sequences for 8t and 9q genes were derived as follows. The partial rat PCIP sequences were used to prepare probes, which were then used to screen, for example, rat mid brain cDNA libraries. Positive clones were identified, amplified and sequenced using standard techniques, to obtain the full length sequence. Additionally, a rapid amplification of the existing rat
10 PCIP cDNA ends (using for example, 5' RACE, by Gibco, BRL) was used to complete the 5' end of the transcript.

EXAMPLE 2: IDENTIFICATION OF HUMAN 1v cDNA

 To obtain the human 1v nucleic acid molecule, a cDNA library made from a
15 human hippocampus (Clontech, Palo Alto, CA) was screened under low stringency conditions as follows: Prehybridization for 4 hours at 42°C in Clontech Express Hyb solution, followed by overnight hybridization at 42°C. The probe used was a PCR-generated fragment including nucleotides 49-711 of the rat sequence labeled with ³²P dCTP. The filters were washed 6 times in 2XSSC/0.1% SDS at 55°C. The same
20 conditions were used for secondary screening of the positive isolates. Clones thus obtained were sequenced using an ABI automated DNA Sequencing system, and compared to the rat sequences shown in SEQ ID NO:3 as well as to known sequences from the GenBank database. The largest clone from the library screen was subsequently subcloned into pBS-KS+ (Stratagene, La Jolla, CA) for sequence verification. The 515
25 base pair clone was determined to represent the human homolog of the 1v gene, encompassing 211 base pairs of 5' UTR and a 304 base pair coding region. To generate the full-length cDNA, 3' RACE was used according to the manufacturers instructions (Clontech Advantage PCR kit).

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EXAMPLE 3: ISOLATION AND CHARACTERIZATION OF 1V SPLICE VARIANTS

The mouse 1v shown in SEQ ID NO:5 and the rat 1vl splice variant shown in SEQ ID NO:7 was isolated using a two-hybrid assay as described in Example 1. The mouse 1vl splice variant shown in SEQ ID NO: 7 was isolated by screening a mouse brain cDNA library, and the rat 1vn splice variant shown in SEQ ID NO:11 was isolated by BLAST searching.

EXAMPLE 4: ISOLATION AND IDENTIFICATION OF 9Q AND OTHER PCIPs

Rat 9ql (SEQ ID NO: 15) was isolated by database mining, rat 9qm (SEQ ID NO: 21) was isolated by a two-hybrid assay, and rat 9qc (SEQ ID NO:27) was identified by database mining. Human 9ql (SEQ ID NO: 13), and human 9qs (SEQ ID NO: 23) were identified as described in Example 2. Mouse 9ql (SEQ ID NO:17), monkey 9qs (SEQ ID NO:25), human p193 (SEQ ID NO:39), rat p19 (SEQ ID NO:33), and mouse p19 (SEQ ID NO:35) were identified by database mining. Rat 8t (SEQ ID NO:29) was identified using a two-hybrid assay. The sequence of W28559 (SEQ ID NO:37) was identified by database mining and sequencing of the identified EST with Genbank Accession Number AI352454. The protein sequence was found to contain a 41 amino acid region with strong homology to 1v, 9ql, and p19 (see alignment in Figure 25). However, downstream of this homologous region the sequence diverges from that of the PCIP family. This sequence could represent a gene which possesses a 41 amino acid domain with homology to a similar domain found in the PCIP family members.

The human genomic 9q sequence (SEQ ID NOs:46 and 47) was isolated by screening a BAC genomic DNA library (Reasearch Genetics) using primers which were designed based on the sequence of the human 9qm cDNA. Two positive clones were identified (448O2 and 721I17) and sequenced.

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EXAMPLE 5: EXPRESSION OF 1V, 8T, AND 9Q mRNA IN RAT TISSUES

Rat and mouse multiple tissue Northern blots (Clontech) were probed with a [32P]-labeled cDNA probe directed at the 5'-untranslated and 5'-coding region of the rat 1v sequence (nucleotides 35-124; SEQ ID NO:3) (this probe is specific for rat 1v and rat 1vl), the 5' coding region of the 8t sequence (nucleotides 1-88; SEQ ID NO:29) (this probe is specific for 8t), or the 5' end of the rat 9qm sequence (nucleotides 1-195; SEQ ID NO:21) (this probe is specific for all 9q isoforms, besides 8t). Blots were hybridize using standard techniques. Northern blots hybridized with the rat 1v probe revealed a single band at 2.3kb only in the lane containing brain RNA, suggesting that 1v expression is brain specific. Northern blots probed with the rat 8t probe revealed a major band at 2.4kb. The rat 8t band was most intense in the lane containing heart RNA and there was also a weaker band in the lane containing brain RNA. Northern blots hybridized with the 9q cDNA probe revealed a major band at 2.5kb and a minor band at over 4kb with predominant expression in brain and heart. The minor band may represent incompletely spliced or processed 9q mRNA. The results from the northern blots further indicated that p19 is expressed predominantly in the heart.

EXAMPLE 6: EXPRESSION OF 1V, 8T, AND 9Q IN BRAIN

Expression of the rat 1v and 8t/9q genes in the brain was examined by *in situ* hybridization histochemistry (ISHH) using [35S]-labeled cRNA probes and a hybridization procedure identical to that described in Rhodes *et al.* (1996) J. Neurosci., 16:4846-4860. Templates for preparing the cRNA probes were generated by standard PCR methods. Briefly, oligonucleotide primers were designed to amplify a fragment of 3'- or 5'-untranslated region of the target cDNA and in addition, add the promoter recognition sequences for T7 and T3 polymerase. Thus, to generate a 300 nucleotide probe directed at the 3'-untranslated region of the 1v mRNA, we used the following primers:

5-TAATACGACTCACTATAGGGACTGGCCATCCTGCTCTCAG-3 (T7, forward, sense; SEQ ID NO:42)

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5-ATTAACCCTCACTAAAGGGCACTACTGTTTAAGCTCAAG-3 (T3, reverse, antisense; SEQ ID NO:43). The underlined bases correspond to the T7 and T3 promoter sequences. To generate a probe directed at a 325 bp region of 3'-untranslated sequence shared by the 8t and 9q mRNAs, the following primers were used:

- 5 5-TAATACGACTCACTATAGGGCACCTCCCCTCCGGCTGTTC-3 (T7, forward, sense; SEQ ID NO:44)
 5-ATTAACCCTCACTAAAGGGGAGAGCAGCAGCATGGCAGGGT-3 (T3, reverse, antisense; SEQ ID NO:45).

Autoradiograms of rat brain tissue sections processed for ISHH localization of 1v
 10 or 8t/9q mRNA expression revealed that 1v mRNA is expressed widely in brain in a pattern consistent with labeling of neurons as opposed to glial or endothelial cells. 1v mRNA is highly expressed in cortical, hippocampal, and striatal interneurons, the reticular nucleus of the thalamus, the medial habenula, and in cerebellar granule cells. 1v mRNA is expressed at moderate levels in midbrain nuclei including the substantia nigra
 15 and superior colliculus, in several other thalamic nuclei, and in the medial septal and diagonal band nuclei of the basal forebrain.

Because the probe used to analyze the expression of 8t and 9q hybridizes to a region of the 3'-untranslated region that is identical in the 8t and 9q mRNAs, this probe generates a composite image that reveals that 8t/9q mRNA is expressed widely in brain
 20 in a pattern that partly overlaps with that for 1v as described above. However, 8t/9q mRNA is highly expressed in the striatum, hippocampal formation, cerebellar granule cells, and neocortex. 8t/9q mRNA is expressed at moderate levels in the midbrain, thalamus, and brainstem. In many of these areas, 8t/9q mRNA appears to be concentrated in interneurons in addition to principal cells, and in all regions 8t/9q
 25 expression appears to be concentrated in neurons as opposed to glial cells.

Single- and double-label immunohistochemistry revealed that the PCIP and Kv4 polypeptides are precisely colocalized in many of the cell types and brain regions where PCIP and Kv4 mRNAs are coexpressed. For example, 9qm colocalized with Kv4.2 in the somata and dendrites of hippocampal granule and pyramidal cells, neurons in the
 30 medial habenular nucleus and in cerebellar basket cells, while 1v colocalized with Kv4.3 in layer II neurons of posterior cingulate cortex, hippocampal interneurons, and in a

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subset of cerebellar granule cells. Immunoprecipitation analyses indicated that 1v and 9qm are coassociated with Kv4 α -subunits in rat brain membranes.

**EXAMPLE 7: CO-ASSOCIATION OF PCIPs AND Kv4 CHANNELS
IN COS AND CHO CELLS**

COS1 and CHO cells were transiently transfected with individual PCIPs (KChIP1, KChIP2, KChIP3) alone or together with Kv4.2 or Kv4.3 using the lipofectamine plus procedure essentially as described by the manufacturer (Boehringer Mannheim). Forty-eight hours after the transfection, cells were washed, fixed, and processed for immunofluorescent visualization as described previously (Bekele-Arcuri *et al.* (1996) *Neuropharmacology*, 35:851-865). Affinity-purified rabbit polyclonal or mouse monoclonal antibodies to the Kv4 channel or the PCIP protein were used for immunofluorescent detection of the target proteins.

When expressed alone, the PCIPs were diffusely distributed throughout the cytoplasm of COS-1 and CHO cells, as would be expected for cytoplasmic proteins. In contrast, when expressed alone, the Kv4.2 and Kv4.3 polypeptides were concentrated within the perinuclear ER and Golgi compartments, with some immunoreactivity concentrated in the outer margins of the cell. When the PCIPs were coexpressed with Kv4 α -subunits, the characteristic diffuse PCIP distribution changed dramatically, such that the PCIPs precisely colocalized with the Kv4 α -subunits. This redistribution of the PCIPs did not occur when they were coexpressed with the Kv1.4 α -subunit, indicating that altered PCIP localization is not a consequence of overexpression and that these PCIPs associate specifically with Kv4-family α -subunits.

To verify that the PCIP and Kv4 polypeptides are tightly associated and not simply colocalized in co-transfected cells, reciprocal immunoprecipitation analyses were performed using the PCIP and channel-specific antibodies described above. All three PCIP polypeptides coassociated with Kv4 α -subunits in cotransfected cells, as evidenced by the ability of anti-Kv4.2 and anti-Kv4.3 antibodies to immunoprecipitate the KChIP1, KChIP2, and KChIP3 proteins from lysates prepared from cotransfected cells, and by the ability of anti-PCIP antibodies to immunoprecipitate Kv4.2 and Kv4.3

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α -subunits from these same lysates. The cells were lysed in buffer containing detergent and protease inhibitors, and prepared for immunoprecipitation reactions essentially as described previously (Nakahira *et al.* (1996) J. Biol. Chem., 271:7084-7089).

Immunoprecipitations were performed as described in Nakahira *et al.* (1996) J. Biol.

5 Chem., 271:7084-7089 and in Harlow E. and Lane, D., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, c1988. The products resulting from the immunoprecipitation were size fractionated by SDS-PAGE and transferred to nitrocellulose filters using standard procedures.

To confirm that the cytoplasmic N-terminus of Kv4 channels is sufficient for the
 10 interaction with the PCIPs KChIP1 or KChIP2 were co-expressed with a Kv4.3 mutant (Kv4.3 Δ C) that lacks the entire 219 amino acid cytoplasmic C-terminal tail. In transiently transfected COS-1 cells, the Kv4.3 Δ C mutant was extensively trapped within the perinuclear ER and Golgi: little or no staining was observed at the outer margins of the cell. Nonetheless, KChIP1 and KChIP2 precisely colocalized with Kv4.3 Δ C in
 15 cotransfected cells, and moreover, Kv4.3 Δ C was efficiently coimmunoprecipitated by PCIP antibodies, indicating that the interaction of these PCIPs with Kv4 α -subunits does not require the cytoplasmic C-terminus of the channel.

20 **EXAMPLE 8: CO-ASSOCIATION OF PCIPs AND Kv4 CHANNELS IN NATIVE TISSUES**

To determine whether PCIPs colocalize and co-associate with Kv4 subunits in native tissues, Kv4- and PCIP-specific antibodies were used for single and double-label immunohistochemical analyses and for reciprocal coimmunoprecipitation analyses of rat brain membranes. Immunohistochemical staining of rat brain sections indicated that
 25 KChIP1 and KChIP2 colocalize with Kv4.2 and Kv4.3 in a region and cell type-specific manner. For example, KChIP1 colocalized with Kv4.3 in hippocampal interneurons, cerebellar granule cells, and cerebellar glomeruli, a specialized synaptic arrangement between the dendrites of cerebellar basket and golgi cells and mossy fiber terminals. KChIP2 colocalized with Kv4.3 and Kv4.2 in the dendrites of granule cells in the
 30 dentate gyrus, in the apical and basal dendrites of hippocampal and neocortical

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pyramidal cells, and in several subcortical structures including the striatum and superior colliculus. Co-immunoprecipitation analyses performed using synaptic membranes prepared from whole rat brain revealed that the PCIPs (KChIPs 1, 2, and 3) are tightly associated with Kv4.2 and Kv4.3 in brain K⁺ channel complexes. Anti-PCIP antibodies immunoprecipitated Kv4.2 and Kv4.3 from brain membranes, and anti-Kv4.2 and Kv4.3 antibodies immunoprecipitated the PCIPs. None of the PCIP polypeptides were immunoprecipitated by anti-Kv2.1 antibodies, indicating that the association of these PCIPs with brain Kv channels may be specific for Kv4 α -subunits. Taken together, these anatomical and biochemical analyses indicate that these PCIPs are integral components of native Kv4 channel complexes.

EXAMPLE 9: PCIPs ARE CALCIUM BINDING PROTEINS

To determine whether KChIPs 1, 2, and 3 bind Ca²⁺, GST-fusion proteins were generated for each PCIP and the ability of the GST-PCIP proteins, as well as the recombinant PCIP polypeptides enzymatically cleaved from GST, to bind ⁴⁵Ca²⁺ was examined using a filter overlay assay (described in, for example, Kobayashi *et al.* (1993) *Biochem. Biophys. Res. Commun.* 189(1):511-7). All three PCIP polypeptides, but not an unrelated GST-fusion protein, display strong ⁴⁵Ca²⁺ binding in this assay. Moreover, all three PCIP polypeptides display a Ca²⁺-dependent mobility shift on SDS-PAGE, indicating that like the other members of this family, KChIPs 1, 2 and 3 are in fact Ca²⁺-binding proteins (Kobayashi *et al.* (1993) *supra*; Buxbaum *et al.* Nef (1996). Neuron-specific calcium sensors (the NCS-1 subfamily). In: Celio MR (ed) *Guidebook to the calcium-binding proteins*. Oxford University Press, New York, pp94-98; Buxbaum J.D., *et al.* (1998) *Nature Med.* 4(10):1177-81.

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EXAMPLE 10: ELECTROPHYSIOLOGICAL CHARACTERIZATION OF PCIPs

Because PCIPs, *e.g.*, KChIP1 (1v), KChIP2 (9ql), and KChIP3 (p19), colocalize and coassociate with Kv4 α -subunits in brain, another critical question was to determine whether these PCIPs alter the conductance properties of Kv4 channels. To address this issue, Kv4.2 and Kv4.3 were expressed alone and in combination with individual PCIPs. CHO cells were transiently-transfected with cDNA using the DOTAP lipofection method as described by the manufacturer (Boehringer Mannheim, Inc.). Transfected cells were identified by cotransfecting enhanced GFP along with the genes of interest and subsequently determining if the cells contained green GFP fluorescence. Currents in CHO cells were measured using the patch-clamp technique (Hamill *et al.* 1981. Pfluegers Arch. 391: 85-100).

Transient transfection of the rat Kv4.2 α -subunit in CHO cells resulted in expression of a typical A-type K⁺ conductance. Coexpression of Kv4.2 with KChIP1 revealed several dramatic effects of KChIP1 on the channel (Figure 41 and Table 1). First, the amplitude of the Kv4.2 current increased approximately 7.5 fold in the presence of KChIP1 (amplitude of Kv4.2 alone = 0.60 +/- 0.096 nA/cell; Kv4.2 + KChIP1 = 4.5 +/- 0.55 nA/cell). When converted into current density by correcting for cell capacitance, a measure of cell surface membrane area, the Kv4.2 current density increased 12 fold with coexpression of KChIP1 (Kv4.2 alone = 25.5 +/- 3.2 pA/pF; Kv4.2 + KChIP1 = 306.9 +/- 57.9 pA/pF), indicating that KChIPs promote and/or stabilize Kv4.2 surface expression. Together with this increase in current density, a dramatic leftward shift in the threshold for activation of Kv4.2 currents was observed in cells expressing Kv4.2 and KChIP1 (activation V_{1/2} for Kv4.2 alone = 20.8 +/- 7.0mV, Kv4.2 + KChIP1 = -12.1 +/- 1.4 mV). Finally, the kinetics of Kv4.2 inactivation slowed considerably when Kv4.2 was coexpressed with KChIP1 (inactivation time constant of Kv4.2 alone = 28.2 +/- 2.6 ms; Kv4.2 + KChIP1 = 104.1 +/- 10.4 ms), while channels recovered from inactivation much more rapidly in cells expressing both Kv4.2 and KChIP1 (recovery tau = 53.6 +/- 7.6 ms) versus cells expressing Kv4.2 alone (recovery tau = 272.2 +/- 26.1 ms).

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KChIPs1, 2 and 3 have distinct N-termini but share considerable amino acid identity within the C-terminal "core" domain. Despite their distinct N-termini, the effects of KChIP2 and KChIP3 on Kv4.2 current density and kinetics were strikingly similar to those produced by KChIP1 (Table1). Thus to confirm that the conserved C-terminal core domain, which contains all three EF-hands, is sufficient to modulate Kv4 current density and kinetics, N-terminal truncation mutants of KChIP1 and KChIP2 were prepared. The KChIP1 Δ N2-31 and KChIP2 Δ N2-67 mutants truncated KChIP1 and KChIP2, respectively, to the C-terminal 185 amino acid core sequence. Coexpression of KChIP1 Δ N2-31 or KChIP2 Δ N2-67 with Kv4.2 in CHO cells produced changes in Kv4.2 current density and kinetics that were indistinguishable from the effects produced by full-length KChIP1 or KChIP2 (Table1).

To investigate whether the modulatory effects of these KChIPs are specific for Kv4 channels, KChIP1 was coexpressed with Kv1.4 and Kv2.1 in *Xenopus* oocytes. *Xenopus* oocytes were injected with 1-3 ng/oocyte of cRNA which was prepared using standard in vitro transcription techniques (Sambrook *et al.* 1989. Molecular Cloning: a laboratory manual, Cold Spring Harbor Press). Currents in oocytes were measured with a two-electrode voltage clamp. KChIP1 did not appear to have any effect on Kv1.4 or Kv2.1 currents (Table2), indicating that these functional effects may be specific for Kv4 channels. As a final control for the KChIP effects and to verify that the KChIPs' effects on Kv4 currents are independent of expression system, the above kinetic analyses were repeated after expressing Kv4.3 and KChIP mRNAs in *Xenopus* oocytes. The effects KChIP1 on for Kv4.3 in the oocyte system were strikingly similar to those on Kv4.2 in CHO cells (Table1).

Since these KChIPs bind Ca²⁺, another important question is to determine whether the effects of KChIP1 on Kv4.2 currents are Ca²⁺-dependent. This question was addressed indirectly by introducing point mutations within each of KChIP1's EF-hand domains: one mutant has point mutations in the first two EF hands (D₁₉₉ to A, G₁₀₄ to A, D₁₃₅ to A, and G₁₄₀ to A) and the other one has point mutations in all three EF hands (D₁₉₉ to A, G₁₀₄ to A, D₁₃₅ to A, G₁₄₀ to A, D₁₈₃ to A, and G₁₈₈ to A). These mutations substituted alanine for the two most highly conserved amino acids within the EF-hand consensus (Figure 25; Linse, S. and Forsen, S. (1995) Determinants that govern high-

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affinity Calcium binding. In Means, S. (Ed.)Advances in second messenger and phosphoprotein research. New York, Ravens Press,. 30:89-150). Coexpression of this KChIP1 triple EF-hand mutant with Kv4.2 or Kv4.3 in COS cells indicated that this mutant colocalizes and is efficiently coimmunoprecipitated with Kv4 α -subunits in

5 COS-1 cells. However, these EF-hand point mutations completely eliminated the effects of KChIP1 on Kv4.2 kinetics (Table1). Taken together, these results indicate that the binding interaction between KChIP1 and Kv4.2 is Ca^{2+} independent, while modulation of Kv4.2 kinetics by KChIP1 is either Ca^{2+} -dependent or sensitive to structural changes induced by point mutations within the EF-hand domains.

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TABLE 1

Functional effect of KchIPs on Kv4 channels

Current Parameter	rKv4.2 + vector	rKv4.2 + KchIP1	rKv4.2 + KchIP1 Δ N2-31	rKv4.2 + KchIP2	rKv4.2 + KchIP2 Δ N2-67	rKv4.2 + KchIP3	rKv4.3	rKv4.3 + KchIP1
Peak Current	0.60*	4.5*	6.0*	3.3*	5.8*	3.5*	7.7 μ A	18.1 μ A*
(nA/cell at 50 mV)	\pm 0.096	\pm 0.055	\pm 1.1	\pm 0.45	\pm 1.1	\pm 0.99	\pm 2.6	\pm 3.8
Peak Current Density	25.5	306.9*	407.2*	196.6*	202.6*	161.7*	---	---
(pA/pF at 50 mV)	\pm 3.2	\pm 57.9	\pm 104.8	\pm 26.6	\pm 27.5	\pm 21.8		
Inactivation time constant	28.2	104.1	129.2	95.1*	109.5*	67.2*	56.3	135.0
(ms, at 50 mV)	\pm 2.6	\pm 10.4	\pm 14.2	\pm 8.3	\pm 9.6	\pm 14.1	\pm 6.6	\pm 15.1
Recovery from Inactivation Time constant	272.2	53.6*	98.1*	49.5*	36.1*	126.1*	327.0	34.5*

* Significantly different from control.

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TABLE 2

Functional effects of KChIPs on other Kv channels

Current Parameter	Oocytes		Oocytes	
	HKv1.4	hKv1.4 + 1v	HKv2.1	HKv2.1 + 1v
Peak Current	8.3	6.5	3.7	2.9
(μ A/cell at 50 mV)	± 2.0	± 0.64	± 0.48	± 0.37
Inactivation time constant	53.2	58.2	1.9 s	1.7 s
(ms, at 50 mV)	± 2.8	± 6.6	± 0.079	0.078
Recovery from Inactivation time constant (sec, at -80 mV)	1.9	1.6	7.6	7.7
Activation $V_{1/2}$ (mV)	-21.0	-20.9	12.0	12.4
Steady-state Inactivation $V_{1/2}$ (mV)	-48.1	-47.5	-25.3	-23.9

**5 EXAMPLE 11: EFFECTS OF KChIP1 ON SURFACE EXPRESSION OF
KV4- α SUBUNITS IN COS-1 CELLS**

To examine the ability of KChIP1 to enhance the surface expression of Kv4 channels, the ability of KChIP1 to promote the formation of surface co-clusters of Kv4 channels and PSD-95 was monitored. PSD-95 is used to facilitate the visualization of

10 the complex.

To facilitate the interaction between Kv4.3 and PSD-95, a chimeric Kv4.3 subunit (Kv4.3ch) was generated in which the C-terminal 10 amino acids from rKv1.4

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(SNAKAVETDV, SEQ ID NO:73) were appended to the C-terminus of Kv4.3. The C-terminal 10 amino acids from rKv1.4 were used because they associate with PSD-95 and confer the ability to associate with PSD-95 to the Kv4.3 protein when fused to the Kv4.3 C-terminus. Expression of Kv4.3ch in COS-1 cells revealed that the Kv4.3ch

5 polypeptide was trapped in the perinuclear cytoplasm, with minimal detectable Kv4.3ch immunoreactivity at the outer margins of the cell. When Kv4.3ch was co-expressed with PSD-95, PSD-95 became trapped in the perinuclear cytoplasm and co-localized with Kv4.3ch. However, when KChIP1 was co-expressed with Kv4.3ch and PSD-95, large plaque-like surface co-clusters of Kv4.3ch, KChIP1 and PSD-95 were observed.

10 Triple-label immunofluorescence confirmed that these surface clusters contain all three polypeptides, and reciprocal co-immunoprecipitation analyses indicated that the three polypeptides are co-associated in these surface clusters. Control experiments indicated that KChIP1 does not interact with PSD-95 alone, and does not co-localize with Kv1.4 and PSD-95 in surface clusters. Taken together, these data indicate that KChIP1 may

15 promote the transit of the Kv4.3 subunits to the cell surface.

EXAMPLE 12: CHARACTERIZATION OF THE PCIP PROTEINS

In this example, the amino acid sequences of the PCIP proteins were compared to amino acid sequences of known proteins and various motifs were identified.

20 The 1v polypeptide, the amino acid sequence of which is shown in SEQ ID NO:3 is a novel polypeptide which includes 216 amino acid residues. Domains that are putatively involved in calcium binding (Linse, S. and Forsen, S. (1995) *Advances in Second Messenger and Phosphoprotein Research* 30, Chapter 3, p89-151, edited by Means, AR., Raven Press, Ltd., New York), were identified by sequence alignment (see

25 Figure 21).

The 8t polypeptide, the amino acid sequence of which is shown in SEQ ID NO:30 is a novel polypeptide which includes 225 amino acid residues. Calcium binding domains that are putatively involved in calcium binding (Linse, S. and Forsen, S. (1995) *Advances in Second Messenger and Phosphoprotein Research* 30, Chapter 3, p89-151, edited by Means, AR., Raven Press, Ltd., New York), were identified by

30 sequence alignment (see Figure 21).

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The 9q polypeptide is a novel polypeptide which includes calcium binding domains that are putatively involved in calcium binding (Linse, S. and Forsen, S. (1995) *Advances in Second Messenger and Phosphoprotein Research* 30, Chapter 3, p89-151, edited by Means, AR., Raven Press, Ltd., New York (see Figure 21).

- 5 The p19 polypeptide is a novel polypeptide which includes calcium binding domains that are putatively involved in calcium binding (Linse, S. and Forsen, S. (1995) *Advances in Second Messenger and Phosphoprotein Research* 30, Chapter 3, p89-151, edited by Means, AR., Raven Press, Ltd., New York (see Figure 21).

- 10 A BLASTN 2.0.7 search (Altschul *et al.* (1990) *J. Mol. Biol.* 215:403) of the nucleotide sequence of rat 1vl revealed that the rat 1vl is similar to the rat cDNA clone RMUAH89 (Accession Number AA849706). The rat 1 vl nucleic acid molecule is 98% identical to the rat cDNA clone RMUAH89 (Accession Number AA849706) over nucleotides 1063 to1488.

- 15 A BLASTN 2.0.7 search (Altschul *et al.* (1990) *J. Mol. Biol.* 215:403) of the nucleotide sequence of human 9ql revealed that the human 9ql is similar to the human cDNA clone 1309405 (Accession Number AA757119). The human 9 ql nucleic acid molecule is 98% identical to the human cDNA clone 1309405 (Accession Number AA757119) over nucleotides 937 to1405.

- 20 A BLASTN 2.0.7 search (Altschul *et al.* (1990) *J. Mol. Biol.* 215:403) of the nucleotide sequence of mouse P19 revealed that the mouse P19 is similar to the Mus musculus cDNA clone MNCb-7005 (Accession Number AU035979). The mouse P19 nucleic acid molecule is 98% identical to the Mus musculus cDNA clone MNCb-7005 (Accession Number AU035979) over nucleotides 1 to 583.

25 **EXAMPLE 13: EXPRESSION OF RECOMBINANT PCIP PROTEINS IN BACTERIAL CELLS**

- In this example, PCIP is expressed as a recombinant glutathione-S-transferase (GST) fusion polypeptide in *E. coli* and the fusion polypeptide is isolated and characterized. Specifically, PCIP is fused to GST and this fusion polypeptide is
30 expressed in *E. coli*, e.g., strain BI21. Expression of the GST-PCIP fusion protein in BI21 is induced with IPTG. The recombinant fusion polypeptide is purified from crude

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bacterial lysates of the induced BI21 strain by affinity chromatography on glutathione beads. Using polyacrylamide gel electrophoretic analysis of the polypeptide purified from the bacterial lysates, the molecular weight of the resultant fusion polypeptide is determined.

5 Rat 1v and 9ql were cloned into pGEX-6p-2 (Pharmacia). The resulting recombinant fusion proteins were expressed in *E. coli* cells and purified following art known methods (described in, for example, *Current Protocols in Molecular Biology*, eds. Ausubel *et al.* John Wiley & Sons: 1992). The identities of the purified proteins were verified by western blot analysis using antibodies raised against peptide epitopes of
10 rat 1v and 9ql.

EXAMPLE 14: EXPRESSION OF RECOMBINANT PCIP PROTEINS IN COS CELLS

To express the PCIP gene in COS cells, the pcDNA/Amp vector by Invitrogen
15 Corporation (San Diego, CA) is used. This vector contains an SV40 origin of replication, an ampicillin resistance gene, an *E. coli* replication origin, a CMV promoter followed by a polylinker region, and an SV40 intron and polyadenylation site. A DNA fragment encoding the entire PCIP protein and an HA tag (Wilson *et al.* (1984) *Cell* 37:767) or a FLAG tag fused in-frame to its 3' end of the fragment is cloned into the
20 polylinker region of the vector, thereby placing the expression of the recombinant protein under the control of the CMV promoter.

To construct the plasmid, the PCIP DNA sequence is amplified by PCR using two primers. The 5' primer contains the restriction site of interest followed by approximately twenty nucleotides of the PCIP coding sequence starting from the
25 initiation codon; the 3' end sequence contains complementary sequences to the other restriction site of interest, a translation stop codon, the HA tag or FLAG tag and the last 20 nucleotides of the PCIP coding sequence. The PCR amplified fragment and the pCDNA/Amp vector are digested with the appropriate restriction enzymes and the vector is dephosphorylated using the CIAP enzyme (New England Biolabs, Beverly,
30 MA). Preferably the two restriction sites chosen are different so that the PCIP gene is inserted in the correct orientation. The ligation mixture is transformed into *E. coli* cells

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(strains HB101, DH5a, SURE, available from Stratagene Cloning Systems, La Jolla, CA, can be used), the transformed culture is plated on ampicillin media plates, and resistant colonies are selected. Plasmid DNA is isolated from transformants and examined by restriction analysis for the presence of the correct fragment.

- 5 COS cells are subsequently transfected with the PCIP-pcDNA/Amp plasmid DNA using the calcium phosphate or calcium chloride co-precipitation methods, DEAE-dextran-mediated transfection, lipofection, or electroporation. Other suitable methods for transfecting host cells can be found in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989. The expression of the PCIP polypeptide is detected by radiolabelling (^{35}S -methionine or ^{35}S -cysteine available from NEN, Boston, MA, can be used) and immunoprecipitation (Harlow, E. and Lane, D. *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988) using an HA specific monoclonal antibody. Briefly, the cells are labelled for 8 hours with ^{35}S -methionine (or ^{35}S -cysteine). The culture media are then collected and the cells are lysed using detergents (RIPA buffer, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM Tris, pH 7.5). Both the cell lysate and the culture media are precipitated with an HA specific monoclonal antibody. Precipitated polypeptides are then analyzed by SDS-PAGE.
- 10
- 15
- 20 Alternatively, DNA containing the PCIP coding sequence is cloned directly into the polylinker of the pCDNA/Amp vector using the appropriate restriction sites. The resulting plasmid is transfected into COS cells in the manner described above, and the expression of the PCIP polypeptide is detected by radiolabelling and immunoprecipitation using a PCIP specific monoclonal antibody.
- 25 Rat 1v was cloned into the mammalian expression vector pRBG4. Transfections into COS cells were performed using LipofectAmine Plus (Gibco BRL) following the manufacturer's instructions. The expressed 1v protein was detected by immunocytochemistry and/or western blot analysis using antibodies raised against 1v in rabbits or mice.

**EXAMPLE 15: IDENTIFICATION AND CHARACTERIZATION OF
HUMAN FULL LENGTH P19**

The human full length p19 sequence was identified using RACE PCR. The sequence of p19 (also referred to as KChIP3) is shown in Figure 16. The amino acid
5 sequence of human p19 is 92% identical to the mouse p19 gene (SEQ ID NO:35).

TBLASTN searches using the protein sequence of human p19 revealed that human p19 is homologous to two sequences, Calsenilin (described in (1998) *Nature Medicine* 4: 1177-1181) and DREAM, a Ca²⁺-dependent regulator of prodynorphin and c-fos transcription (described in Carrion *et al.* (1999) *Nature* 398: 80-84). Human p19 is
10 100% identical at the nucleotide level to Calsenilin (but extends 3' to the published sequence) and 99% identical at the nucleotide level to DREAM.

The ability of p19 (as well as other PCIP family members) to co-localize with presenilin and act as transcription factors is determined using art known techniques such as northern blots, *in situ* hybridization, β -gal assays, DNA mobility assays (described in,
15 for example, Carrion *et al.* (1999) *Nature* 398:80) and DNA mobility supershift assays, using antibodies specific for KchIPs.

Other assays suitable for evaluating the association of PCIP family members with presenilins is co-immunoprecipitation (described in, for example, Buxbaum *et al.* (1998) *Nature Medicine* 4:1177).

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**EXAMPLE 16: IDENTIFICATION AND CHARACTERIZATION OF
MONKEY KChIP4**

In this example, the identification and characterization of the genes encoding monkey KChIP4a (jlkbd352e01t1) and alternatively spliced monkey KChIP4b
25 (jlkbb231c04t1), KChIP4c (jlksa053c02), and KChIP4d (jlkx015b10) is described.

TBLASTN searches in proprietary databases with the sequence of the known PCIP family members, lead to the identification of four clones jlkbb231c04t1, jlkbd352e01t1, jlksa053c02, and jlkx015b10. The four monkey clones were obtained and sequenced.

The sequences of proprietary monkey clones jlkbb231c04t1 and jlkbd352e01t1
30 were found to correspond to alternately spliced variants of an additional PCIP family member, referred to herein as KChIP4. Clone jlkbb231c04t1 contains a 822bp deletion

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relative to jlkbd352e01t1 (presumably due to splicing out of an exon), resulting in the loss of the final EF hand domain. In clone jlkbd352e01t1, the final EF hand domain is preserved, and the C-terminus is highly homologous to that of PCIP family members 1v, 9ql, and p19. Overall identity in the homologous C-termini among KChIP4, 1v, 9ql, and p19 ranged from 71%-80% at the amino acid level (alignments were performed using the CLUSTALW).

Monkey KChIP4c and KChIP4d were discovered by BLASTN search using monkey KChIP4a as a query for searching a proprietary database.

The nucleotide sequence of the monkey KChIP4a cDNA and the predicted amino acid sequence of the KChIP4a polypeptide are shown in Figure 23 and in SEQ ID NOs:48 and 49, respectively.

The nucleotide sequence of the monkey KChIP4b cDNA and the predicted amino acid sequence of the KChIP4b polypeptide are shown in Figure 24 and in SEQ ID NOs:50 and 51, respectively.

The nucleotide sequence of the monkey KChIP4c cDNA and the predicted amino acid sequence of the KChIP4c polypeptide are shown in Figure 35 and in SEQ ID NOs:69 and 70, respectively.

The nucleotide sequence of the monkey KChIP4d cDNA and the predicted amino acid sequence of the KChIP4d polypeptide are shown in Figure 36 and in SEQ ID NOs:71 and 72, respectively.

Figure 37 depicts an alignment of the protein sequences of KChIP4a, KChIP4b, KChIP4c, and KChIP4d.

Rat KChIP4 is predominantly expressed in the brain, and weakly in the kidney, but not in the heart, brain, spleen, lung, liver, skeletal muscle or testes, as indicated by northern blot experiments in which a northern blot purchased from Clontech was probed with a DNA fragment from the 3'-untranslated region of rat KChIP4.

EXAMPLE 17: IDENTIFICATION AND CHARACTERIZATION OF HUMAN AND RAT 33b07

In this example, the identification and characterization of the genes encoding rat and human 33b07 is described. Partial rat 33b07 (clone name 9o) was isolated as a

positive clone from the yeast two-hybrid screen described above, using rKv4.3N as bait. The full length rat 33b07 clone was identified by mining of proprietary databases.

The nucleotide sequence of the full length rat 33b07 cDNA and the predicted amino acid sequence of the rat 33b07 polypeptide are shown in Figure 26 and in SEQ ID NOs:52 and 53, respectively. The rat 33b07 cDNA encodes a protein having a molecular weight of approximately 44.7 kD and which is 407 amino acid residues in length.

Rat 33b07 binds rKv4.3N and rKv4.2N with slight preference for rKv4.2N in yeast 2-hybrid assays. In contrast, rat 33b07 does not bind rKv1.1N, indicating that the rat 33b07-Kv4N interaction is specific.

Rat 33b07 is expressed predominantly in the brain as determined by northern blot analysis.

The human 33b07 ortholog (clone 106d5) was also identified by mining of proprietary databases. The nucleotide sequence of the full length human 33b07 cDNA and the predicted amino acid sequence of the human 33b07 polypeptide are shown in Figure 27 and in SEQ ID NOs:54 and 55, respectively. The human 33b07 cDNA encodes a protein having a molecular weight of approximately 45.1 kD and which is 414 amino acid residues in length.

Human 33b07 is 99% identical to the human KIAA0721 protein (GenBank Accession Number: AB018264) at the amino acid level. However, GenBank Accession Number: AB018264 does not have a functional annotation. Human 33b07 is also homologous to Testes-specific (Y-encoded) proteins (TSP(Y)s), SET, and Nucleosome Assembly Proteins (NAPs). The human 33b07 is 38% identical to human SET protein (GenBank Accession Number Q01105=U51924) over amino acids 204 to 337 and 46% identical over amino acids 334 to 387.

Human SET is also called HLA-DR associated protein II (PHAPII) (Hoppe-Seyler (1994) *Biol. Chem.* 375:113-126) and in some cases is associated with acute undifferentiated leukemia (AUL) as a result of a translocation event resulting in the formation of a SET-CAN fusion gene (Von Lindern M. *et al.* (1992) *Mol. Cell. Biol.* 12:3346-3355). An alternative spliced form of SET is also called Template Activating Factor-I alpha (TAF). TAF is found to be associated with myeloid leukemogenesis

(Nagata K. *et al.* (1995) *Proc. Natl. Acad. Sci. U.S.A.* 92 (10), 4279-4283). Human SET is also a potent protein inhibitor of phosphatase 2A (Adachi Y. *et al.* (1994) *J. Biol. Chem.* 269:2258-2262). NAPs may be involved in modulating chromatin formation and contribute to regulation of cell proliferation (Simon H.U. *et al.* (1994) *Biochem. J.* 297, 389-397).

Thus, due to its homology to the above identified proteins, 33b07 may function as a protein inhibitor of phosphatase, an oncogene, and/or a chromatin modulator. The homology of 33b07 to SET, a protein phosphatase inhibitor, is of particular interest. Many channels, in particular the Kv4 channels (with which 33b07 is associated), are known to be regulated by phosphorylation by PKC and PKA ((1998) *J. Neuroscience* 18(10): 3521-3528; *Am J Physiol* 273: H1775-86 (1997)). Thus, 33b07 may modulate Kv4 activity by regulating the phosphorylation status of the potassium channel.

EXAMPLE 18: IDENTIFICATION AND CHARACTERIZATION OF RAT 1p

In this example, the identification and characterization of the gene encoding rat 1p is described. Partial rat 1p was isolated as a positive clone from the yeast two-hybrid screen described above, using rKv4.3N as a bait.

The nucleotide sequence of the partial length rat 1p cDNA and the predicted amino acid sequence of the rat 1p polypeptide are shown in Figure 28 and in SEQ ID NOs:56 and 57, respectively. The rat 1p cDNA encodes a protein having a molecular weight of approximately 28.6 kD and which is 267 amino acid residues in length.

Rat 1p binds rKv4.3N and rKv4.2N with slight preference for rKv4.3N in yeast two-hybrid assays. In contrast, 1p does not bind rKv1.1N, indicating that the 1p-Kv4N interaction is specific.

Rat 1p is predominantly expressed in the brain as determined by northern blot analysis.

A BLASTP 1.4 search, using a score of 100 and a word length of 3 (Altschul *et al.* (1990) *J. Mol. Biol.* 215:403) of the amino acid sequences of rat 1p revealed that rat 1p is similar to the human Restin (GenBank Accession Number P30622; also named cytoplasmic linker protein-170 alpha-2 (CLIP-170), M97501)). The rat 1p protein is

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58% identical to the human Restin over amino acid residues 105 to 182, 55% identical to the human Restin over amino acid residues 115 to 186, 22% identical to the human Restin over amino acid residues 173 to 246, 22% identical to the human Restin over amino acid residues 169 to 218, and 58% identical to the human Restin over amino acid residues 217 to 228.

Restin is also named Reed-Sternberg intermediate filament associated protein. Reed-Sternberg cells are the tumoral cells diagnostic for Hodgkin's disease. It is suggested that Restin overexpression may be a contributing factor in the progression of Hodgkin's disease (Bilbe G. *et al.* (1992) *EMBO J.* 11: 2103-13) and Restin appears to be an intermediate filament associated protein that links endocytic vesicles to microtubules (Pierre P, *et al.* (1992) *Cell* 70 (6), 887-900).

The cytoskeleton regulates the activity of potassium channels (see, for example, Honore E, *et al.* (1992) *EMBO J.* 11:2465-2471 and Levin G, *et al.* (1996) *J. Biol. Chem.* 271:29321-29328), as well as the activity of other channels, *e.g.*, Ca⁺⁺ channels (Johnson B.D. *et al.* (1993) *Neuron* 10:797-804); or Na⁺ channels (Fukuda J. *et al.* (1981) *Nature* 294:82-85).

Accordingly, based on its homology to the Restin protein, the rat 1p protein may be associated with the cytoskeleton and may modulate the activity of potassium channels, *e.g.*, Kv4, via its association to the cytoskeleton.

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EXAMPLE 19: IDENTIFICATION AND CHARACTERIZATION OF RAT 7s

In this example, the identification and characterization of the gene encoding rat 7s is described. Partial rat 7s was isolated as a positive clone from the yeast two-hybrid screen described above, using rKv4.3N as a bait. Rat 7s is the rat ortholog of the human vacuolar H(+)-ATPase catalytic subunit A (Accession Number P38606 and B46091) described in, for example, van Hille B. *et al.* (1993) *J. Biol. Chem.* 268 (10), 7075-7080.

The nucleotide sequence of the partial length rat 7s cDNA and the predicted amino acid sequence of the rat 7s polypeptide are shown in Figure 29 and in SEQ ID NOs:58 and 59, respectively. The rat 7s cDNA encodes a protein having a molecular weight of approximately 28.6 kD and which is 270 amino acid residues in length.

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Rat 7s binds rKv4.3N and rKv4.2N with preference for rKv4.3N in yeast two-hybrid assays. In contrast, 7s does not bind rKv1.1N, indicating that the 7s-Kv4N interaction is specific.

Rat 7s is expressed at significantly higher levels in the brain and the kidney than in the lung, liver, heart, testes, and skeletal muscle, as determined by northern blot analysis.

EXAMPLE 20: IDENTIFICATION AND CHARACTERIZATION OF RAT 29x AND 25r

In this example, the identification and characterization of the gene encoding rat 29x is described. Rat 29x was isolated as a positive clone from the yeast two-hybrid screen described above, using rKv4.3N as a bait. Rat 25r is a splice variant of 29x. They differ in the 5' untranslated region, but are identical in the coding region and at the amino acid level.

The nucleotide sequence of the rat 29x cDNA and the predicted amino acid sequence of the rat 29x polypeptide are shown in Figure 30 and in SEQ ID NOs:60 and 61, respectively. The rat 29x cDNA encodes a protein having a molecular weight of approximately 40.4 kD and which is 351 amino acid residues in length.

The nucleotide sequence of the rat 25r cDNA is shown in Figure 31 and in SEQ ID NO:62. The rat 25r cDNA encodes a protein having a molecular weight of approximately 40.4 kD and which is 351 amino acid residues in length.

Rat 29x is expressed in the spleen, lung, kidney, heart, brain, testes, skeletal muscle and liver, with the highest level of expression being in the spleen and the lowest being in the liver.

Rat 29x binds rKv4.3N and rKv4.2N with slight preference for rKv4.3N in yeast two-hybrid assays. In contrast, 29x does not bind rKv1.1N, indicating that the 29x-Kv4N interaction is specific.

Rat 29x is identical at the amino acid level to rat SOCS-1 (Suppressor Of Cytokine Signaling) described in Starr R. *et al.* (1997) *Nature* 387: 917-921; to JAB described in Endo T.A. *et al.* (1997) *Nature* 387: 921-924; and to SSI-1 (STAT-induced STAT inhibitor-1) described in Naka T. *et al.* (1997) *Nature* 387:924-928. These

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proteins are characterized in that they have an SH2 domain, bind to and inhibit JAK kinase, and, as a result, regulate cytokine signaling.

As used herein, the term “SH2 domain”, also referred to a Src Homology 2 domain, includes a protein domain of about 100 amino acids in length which is involved in binding of phosphotyrosine residues, *e.g.*, phosphotyrosine residues in other proteins. The target site is called an SH2-binding site. The SH2 domain has a conserved 3D structure consisting of two alpha helices and six to seven beta-strands. The core of the SH2 domain is formed by a continuous beta-meander composed of two connected beta-sheets (Kuriyan J. *et al.* (1997) *Curr. Opin. Struct. Biol.* 3:828-837). SH2 domains function as regulatory modules of intracellular signaling cascades by interacting with high affinity to phosphotyrosine-containing target peptides in a sequence-specific and strictly phosphorylation-dependent manner (Pawson T. (1995) *Nature* 373:573-580). Some proteins contain multiple SH2 domains, which increases their affinity for binding to phosphoproteins or confers the ability to bind to different phosphoproteins. Rat 29x contains an SH2 domain at amino acid residues 219-308 of SEQ ID NO:61.

Tyrosine phosphorylation regulates potassium channel activity (Prevorskaya N.B. *et al.* (1995) *J. Biol. Chem.* 270:24292-24299). JAK kinase phosphorylates proteins at tyrosines and is implicated in the regulation of channel activity (Prevorskaya N.B. *et al. supra*). Accordingly, based on its homology to SOCS-1, JAB, and SSI-1, rat 29x may modulate the activity of potassium channels, *e.g.*, Kv4, by modulating JAK kinase activity.

EXAMPLE 21: IDENTIFICATION AND CHARACTERIZATION OF RAT 5p

In this example, the identification and characterization of the gene encoding rat 5p is described. Rat 5p was isolated as a positive clone from the yeast two-hybrid screen described above, using rKv4.3N as a bait.

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The nucleotide sequence of the rat 5p cDNA and the predicted amino acid sequence of the rat 5p polypeptide are shown in Figure 32 and in SEQ ID NOs:63 and 64, respectively. The rat 5p cDNA encodes a protein having a molecular weight of approximately 11.1 kD and which is 95 amino acid residues in length.

5 Rat 5p binds rKv4.3N and rKv4.2N with similar strength in yeast two-hybrid assays. In contrast, 5p does not bind rKv1.1N, indicating that the 5p-Kv4N interaction is specific.

Rat 5p is expressed in the spleen, lung, skeletal muscle, heart, kidney, brain, liver, and testes, as determined by northern blot analysis.

10 The rat 5p is identical to rat Calpactin I light chain or P10 (Accession Number P05943). P10 binds and induces the dimerization of annexin II (p36). P10 may function as a regulator of protein phosphorylation in that the p36 monomer is the preferred target of a tyrosine-specific kinase (Masiakowski P. *et al.* (1998) *Proc. Natl. Acad. Sci. U.S.A.* 85 (4): 1277-1281).

15 Tyrosine phosphorylation regulates the activity of potassium channels (Prevarskaya N.B. *et al. supra*). Thus, due to its identity to P10, rat 5p may modulate the activity of potassium channels, *e.g.*, Kv4, by modulating the activity of a tyrosine-specific kinase.

20 **EXAMPLE 22: IDENTIFICATION AND CHARACTERIZATION OF RAT 7q**

In this example, the identification and characterization of the gene encoding rat 7q is described. Rat 7q was isolated as a positive clone from the yeast two-hybrid screen described above, using rKv4.3N as a bait. Full length rat 7q was obtained by RACE
25 PCR.

The nucleotide sequence of the rat 7q cDNA and the predicted amino acid sequence of the rat 7q polypeptide are shown in Figure 33 and in SEQ ID NOs:65 and 66, respectively. The rat 7q cDNA encodes a protein having a molecular weight of approximately 23.5 kD and which is 212 amino acid residues in length.

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Rat 7q binds rKv4.3N and rKv4.2N with same strength in yeast two-hybrid assays. In contrast, 7q does not bind rKv1.1N, indicating that the 7q-Kv4N interaction is specific.

Rat 7q is expressed in the heart, brain, spleen, lung, liver, skeletal muscle,
5 kidney, and testes, as determined by northern blot analysis.

Rat 7q is identical to RAB2 (rat RAS-related protein, Accession Number P05712) at the amino acid level. RAB2 appears to be involved in vesicular traffic and protein transport (Touchot N. *et al.* (1987) *Proc. Natl. Acad. Sci. U.S.A.* 84 (23): 8210-8214). Accordingly, based on its homology to RAB2, rat 7q may be involved in
10 potassium channel, *e.g.*, Kv4, trafficking.

EXAMPLE 23: IDENTIFICATION AND CHARACTERIZATION OF RAT 19r

In this example, the identification and characterization of the gene encoding rat
15 19r is described. Partial rat 19r was isolated as a positive clone from the yeast two-hybrid screen described above, using rKv4.3N as a bait. Full length rat 19r was obtained by RACE PCR.

The nucleotide sequence of the rat 19r cDNA and the predicted amino acid sequence of the rat 19r polypeptide are shown in Figure 34 and in SEQ ID NOs:67 and
20 68, respectively. The rat 19r cDNA encodes a protein having a molecular weight of approximately 31.9 kD and which is 271 amino acid residues in length.

Rat 19r is expressed in the heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testes, as determined by northern blot analysis.

Rat 19r binds rKv4.3N and rKv4.2N with slight preference for rKv4.3N in yeast
25 two-hybrid assays. In contrast, 19r does not bind rKv1.1N, indicating that the 19r-Kv4N interaction is specific.

Rat 19r is identical to Rat phosphatidylinositol (PTDINS) transfer protein alpha (PTDINSTP, Accession Number M25758 or P16446) described in Dickeson S.K. *et al.* (1989) *J. Biol. Chem.* 264:16557-16564. PTDINSTP is believed to be involved in
30 phospholipase C-beta (PLC-beta) signaling, phosphatidylinositol transfer protein (PtdIns-TP) synthesis, secretory vesicle formation, and enhancement of

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phosphatidylinositol 3-kinase (PtdIns 3-kinase) activity (Cunningham E. *et al.* (1995) *Curr. Biol.* 5 (7): 775-783; (1995) *Nature* 377 (6549): 544-547; and Panaretou C. *et al.* (1997) *J. Biol. Chem.* 272 (4): 2477-2485).

Accordingly, based on its homology with PTDINSTP, rat 19r may modulate
 5 potassium channel, *e.g.*, Kv4, activity via the PLC-beta signaling pathway and/or the
 PtdIns 3-kinase signaling pathway. Rat p19r may also be involved in potassium
 channel, *e.g.*, Kv4, trafficking.

EXAMPLE 24: CHROMOSOMAL LOCALIZATION OF HUMAN 9q

10 In this example, the human PCIP 9q was chromosomally mapped using a
 radiation hybrid panel (Panel GB4). h9q mapped to a region of chromosome 10q that
 had been previously shown to contain a linkage with partial epilepsy, namely D10S192:
 10q22-q24 (Ottman *et al.* (1995) *Nature Genetics* 10:56-60) (see Figure 43). Based on
 this observation, the present invention clearly demonstrates that the 9q family of
 15 proteins can serve as targets for developing anti-epilepsy drugs and as targets for
 medical intervention of epilepsy.

Furthermore, h9q mapped to a region of chromosome 10q that had been
 previously shown to contain a linkage with IOSCA, namely D10S192 and D10S1265:
 10q24- Nikali (Genomics 39:185-191 (1997)) (see Figures 42 and 43). Based on this
 20 observation, the present invention clearly demonstrates that the 9q family of proteins can
 serve as targets for developing anti-spinocerebellar ataxia drugs and as targets for
 medical intervention of spinocerebellar ataxia.

EXAMPLE 25: KINETIC MODULATION OF KV4-CURRENT BY 25 ARACHIDONIC ACID IS DEPENDENT ON K-CHANNEL INTERACTING PROTEINS

The voltage-gated fast-inactivating Kv4 potassium channels are thought to
 underlie the dendritic A-current in central neurons and the transient outward current (I_{to})
 in cardiac myocytes activating at subthreshold membrane potentials. It has been
 30 reported that arachidonic acid (AA) inhibits both the current formed by Kv4 alpha

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subunits in heterologous cells and the A-current from macropatches excised from hippocampal neurons. However, actions of AA differ in that the neuronal inhibition was associated with kinetic changes that were absent *in vitro*. Most strikingly, the rate of inactivation was considerably increased upon AA application.

- 5 As indicated above, current formed by the Kv4/KChIP combination *in vitro* resembles in many aspects the A-current or I_{to} recorded *in vivo*. In this Example, the role of KChIPs in the kinetic modulation of Kv4-current by arachidonic acid is investigated using art known techniques (described in, for example, An *et al.* (2000) *Nature* 403:553-6; Keros, S. and McBain, C. J. (1997) *J. Neuroscience* 17: 3476-87; and
- 10 Villarroel, A. and Schwarz, T. L. (1996) *J. Neuroscience* 16:2522-32). In Oocytes and in CHO cells, AA inhibited peak amplitude of Kv4 independent of KChIP1. In contrast, perfusion of 10 mM of AA resulted in faster inactivation of Kv4 co-expressed with KChIP1, but did not change the rate of inactivation of Kv4 expressed alone. Thus, the AA effect on inactivation of Kv4/KChIP1 *in vitro* mimics that of the A-current in
- 15 excised neuronal patches. Taken together with the results reported above, these data support the notion that KChIPs are Kv4 auxiliary subunits and that kinetic modulation of Kv4 by AA is dependent on the presence of KChIPs.

20 **EXAMPLE 26: K-CHANNEL INTERACTING PROTEIN-2 (KChIP2)
SPICE VARIANTS, CHROMOSOMAL ORGANIZATION
AND LOCALIZATION**

- In the present Example, variants of KChIP2 and their chromosomal organization were identified using standard techniques. KChIP2 genes are highly conserved at the
- 25 amino acid level among human, rat, and mouse. Multiple human splice variants were identified by database mining and cDNA library screening. Alternative splicing gives rise to N-terminal domains that are variable in length, but the core C-terminal domain is sufficient for associating with and modulating Kv4. The human KChIP2 gene spans approximately 18 kb in the q23 region of human chromosome 10 between WI-8488 and
- 30 WI-6750. This region is syntenic to mouse chromosome 19 between D19Mit40 and D19Mit11. A rat variant discovered by database mining changed the last five amino acids and maintained its ability to associate with and modulate Kv4. Therefore, these multiple variants of KChIP2 appear to function similarly in Kv4 modulation.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

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What is claimed is:

1. An isolated nucleic acid molecule selected from the group consisting of:

5 a) a nucleic acid molecule comprising a nucleotide sequence which is at least 60% identical to the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316, or a complement thereof;

10 b) a nucleic acid molecule comprising a fragment of at least 583 nucleotides of a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316, or a complement thereof;

20 c) a nucleic acid molecule which encodes a polypeptide comprising an amino acid sequence at least about 60% identical to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID

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e) a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72 or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316, under stringent conditions.

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2. The isolated nucleic acid molecule of claim 1 which is selected from the group consisting of:

5 a) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or the DNA
10 insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316, or a complement thereof; and

15 b) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ
20 ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72 or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316.

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3. The nucleic acid molecule of claim 1 further comprising vector nucleic acid sequences.

4. The nucleic acid molecule of claim 1 further comprising nucleic acid
30 sequences encoding a heterologous polypeptide.

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5. A host cell which contains the nucleic acid molecule of claim 1.
6. The host cell of claim 5 which is a mammalian host cell.
- 5 7. A non-human mammalian host cell containing the nucleic acid molecule of claim 1.
8. An isolated polypeptide selected from the group consisting of:
 - 10 a) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, 15 SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72 or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 20 98991, 98993, 98994, or PTA-316, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, 25 SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72 or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 30 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316;

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b) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72 or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316 under stringent conditions; and

c) a polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 60% identical to a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or the DNA insert of the plasmid deposited

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with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316.

5 d) a polypeptide comprising an amino acid sequence which is at least 60% identical to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72 or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316.

15 9. The isolated polypeptide of claim 8 comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72 or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316.

10. The polypeptide of claim 8 further comprising heterologous amino acid sequences.

30 11. An antibody which selectively binds to a polypeptide of claim 8.

12. A method for producing a polypeptide selected from the group consisting of:

a) a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72 or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316;

b) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72 or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72 or an amino acid sequence encoded by the DNA insert of the

plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316; and

- c) a naturally occurring allelic variant of a polypeptide comprising
- 5 the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:49, SEQ ID NO:51, SEQ
- 10 ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:70, or SEQ ID NO:72 or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316, wherein the
- 15 polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:69, or SEQ ID NO:71, or the DNA insert of the plasmid deposited with ATCC as
- 20 Accession Number 98936, 98937, 98938, 98939, 98940, 98941, 98942, 98943, 98944, 98945, 98946, 98947, 98948, 98949, 98950, 98951, 98991, 98993, 98994, or PTA-316 under stringent conditions;
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comprising culturing the host cell of claim 5 under conditions in which the nucleic acid molecule is expressed.

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13. A method for detecting the presence of a polypeptide of claim 8 in a sample comprising:

- 5 a) contacting the sample with a compound which selectively binds to the polypeptide; and
- b) determining whether the compound binds to the polypeptide in the sample to thereby detect the presence of a polypeptide of claim 8 in the sample.

14. The method of claim 13, wherein the compound which binds to the
10 polypeptide is an antibody.

15. A kit comprising a compound which selectively binds to a polypeptide of claim 8 and instructions for use.

15 16. A method for detecting the presence of a nucleic acid molecule in claim 1
in a sample comprising:

- 20
- a) contacting the sample with a nucleic acid probe or primer which selectively hybridizes to the nucleic acid molecule; and
 - b) determining whether the nucleic acid probe or primer binds to a nucleic acid molecule in the sample to thereby detect the presence of a nucleic acid molecule of claim 1 in the sample.

17. The method of claim 16, wherein the sample comprises mRNA molecules and is contacted with a nucleic acid probe.

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18. A kit comprising a compound which selectively hybridizes to a nucleic acid molecule of claim 1 and instructions for use.

19. A method for identifying a compound which binds to a polypeptide of claim 8 comprising:

- a) contacting the polypeptide, or a cell expressing the polypeptide with a test compound; and
- 5 b) determining whether the polypeptide binds to the test compound.

20. The method of claim 19, wherein the binding of the test compound to the polypeptide is detected by a method selected from the group consisting of:

- 10 a) detection of binding by direct detection of test compound/polypeptide binding;
- b) detection of binding using a competition binding assay; and
- c) detection of binding using an assay for PCIP activity.

21. A method for modulating the activity of a polypeptide of claim 8
15 comprising contacting the polypeptide or a cell expressing the polypeptide with a compound which binds to the polypeptide in a sufficient concentration to modulate the activity of the polypeptide.

22. A method for identifying a compound which modulates the activity of a
20 polypeptide of claim 8 comprising:

- a) contacting a polypeptide of claim 8 with a test compound; and
- b) determining the effect of the test compound on the activity of the polypeptide to thereby identify a compound which modulates the activity of the polypeptide.

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23. A method for identifying a compound capable of treating a disorder characterized by aberrant PCIP nucleic acid expression or PCIP protein activity comprising assaying the ability of the compound or agent to modulate the expression of the PCIP nucleic acid molecule of claim 1 or the activity of the PCIP polypeptide of
30 claim 8, thereby identifying a compound capable of treating a disorder characterized by aberrant PCIP nucleic acid expression or PCIP protein activity.

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25. The method of claim 24, wherein the disorder is epilepsy.

27. The method of claim 24, wherein the disorder is spinocerebellar ataxia.

28. The method of claim 23, wherein the disorder is a cardiovascular disorder.

29. The method of claim 28, wherein the cardiovascular disorder is associated with an abnormal I_{to} current.

30. A method for determining if a subject is at risk for a disorder
15 characterized by aberrant or abnormal PCIP nucleic acid expression and/or PCIP protein
activity comprising detecting, in a sample of cells from the subject, the presence or
absence of a genetic lesion, wherein the genetic lesion is characterized by an alteration
affecting the integrity of a gene encoding the PCIP polypeptide of claim 8 or
misexpression of the PCIP nucleic acid molecule of claim 1.

31. The method of claim 30, wherein the disorder is a CNS disorder.

32. The method of claim 31, wherein the disorder is epilepsy.

25 33. The method of claim 31, wherein the disorder is spinocerebellar ataxia.

34. The method of claim 30, wherein the disorder is a cardiovascular disorder.

30 35. The method of claim 34, wherein the cardiovascular disorder is
associated with an abnormal I_{to} current.

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36. A method for identifying a subject suffering from a disorder characterized by aberrant or abnormal PCIP nucleic acid expression and/or PCIP protein activity comprising obtaining a biological sample from the subject, and detecting in the sample, the presence or absence of a genetic lesion, wherein the genetic lesion is characterized by an alteration affecting the integrity of a gene encoding the PCIP polypeptide of claim 8 or misexpression of the PCIP nucleic acid molecule of claim 1, thereby identifying a subject suffering from a disorder characterized by aberrant or abnormal PCIP nucleic acid expression and/or PCIP protein activity.
37. The method of claim 36, wherein the disorder is a CNS disorder.
38. The method of claim 37, wherein the disorder is epilepsy.
39. The method of claim 37, wherein the disorder is spinocerebellar ataxia.
40. The method of claim 36, wherein the disorder is a cardiovascular disorder.
41. The method of claim 40, wherein the cardiovascular disorder is associated with an abnormal I_{to} current.
42. A method for treating a subject having a potassium channel associated disorder comprising administering to the subject a PCIP polypeptide of claim 8 or portion thereof such that treatment occurs.
43. The method of claim 42, wherein the disorder is a CNS disorder.
44. The method of claim 43, wherein the disorder is epilepsy.
45. The method of claim 43, wherein the disorder is spinocerebellar ataxia.

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46. The method of claim 42, wherein the disorder is a cardiovascular disorder.

5 47. The method of claim 46, wherein the cardiovascular disorder is associated with an abnormal I_{to} current.

48. A method for treating a subject having a potassium channel associated disorder comprising administering to the subject a nucleic acid encoding a PCIP
10 polypeptide of claim 8 or portion thereof such that treatment occurs.

49. The method of claim 48, wherein the disorder is a CNS disorder.

50. The method of claim 49, wherein the disorder is epilepsy.
15

51. The method of claim 49, wherein the disorder is spinocerebellar ataxia.

52. The method of claim 48, wherein the disorder is a cardiovascular disorder.
20

53. The method of claim 52, wherein the cardiovascular disorder is associated with an abnormal I_{to} current.

54. Use of the compound identified in the method of claim 23 to treat a
25 potassium channel associated disorder.

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POTASSIUM CHANNEL INTERACTORS AND USES THEREFOR**Abstract of the Disclosure**

- The invention provides isolated nucleic acids molecules, designated PCIP
- 5 nucleic acid molecules, which encode proteins that bind potassium channels and modulate potassium channel mediated activities. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing PCIP nucleic acid molecules, host cells into which the expression vectors have been introduced, and nonhuman transgenic animals in which a PCIP gene has been introduced or disrupted.
- 10 The invention still further provides isolated PCIP proteins, fusion proteins, antigenic peptides and anti-PCIP antibodies. Diagnostic methods utilizing compositions of the invention are also provided.

HUMAN IV DNA (CD:225-87)

[illegible]

HUMAN IV PROTEIN

YYPVLKEDTPRQHVDFVFFQKMDKNKDGIIVTLDEFLESCQEDDNIIMRSLQLFQNVN

Fig. 1

RAT 1vN (r1vN) DNA (CD: 339-1037)

GGCACACAACCCCTGGATTCTTCGGAGAATATGCCGTGAGGTGTTGCCAATTATTAGTTCTCTTGGCTAGCAGATGTTTA
GGGACTGGTtaaGCCTTTGGAGAAATTACCTTAGGAAAACGGGGAAATAAAAGCAAAGATTACCATGAATTGCAAGATTA
CCTAGCAATTGCAAGGtagGAGGAGAGAGGTGGAGGGCGGAGTAGACAGGAGGGAGGGAGAAAGtgaGAGGAAGCTAGGC
TGGTGGAAATAACCTGCACTTGAACAGCGGCAAAGAAGCGCGATTTTCCAGCTTtaaATGCCTGCCCCGCTTCTGCTT
GCCTACCCGGGAACGGAGATGTTGACCCAGGGCGAGTCTGAAGGGCTCCAGACCTTGGGGATAGTAGTGGTCCTGTGTTC
CTCTCTGAACTACTGCACTACCTCGGGCTGATTGACTTGTGCGATGACAAGATCGAGGATGATCTGGAGATGACCATGG
TTTGCCATCGGCCTGAGGGACTGGAGCAGCTTGAGGCACAGACGAACTTCACCAAGAGAGAACTGCAAGTCCTTTACCGG
GGATTCAAAAACGAGTGCCCCAGTGGTGTGGTTAACGAAGAGACATTCAAGCAGATCTACGCTCAGTTTTTCCCTCATGG
AGATGCCAGCACATACGCACATTACCTCTTCAATGCCTTCGACACCACCCAGACAGGCTCTGTAAAGTTCGAGGACTTTG
TGACTGCTCTGTGATTTTACTGAGAGGAACGGTCCATGAAAAACTGAGGTGGACGTTTAATTTGTACGACATCAATAAA
GACGGCTACATAAAACAAAGAGGAGATGATGGACATAGTGAAAGCCATCTATGACATGATGGGGAAATACACCTATCCTGT
GCTCAAAGAGGACACTCCCAGGCAGCACGTGGACGTCTTCTTCCAGAAAATGGATAAAAAATAAGATGGCATTGTAACTG
TAGACGAATTTCTCGAGTCTGTGCTCAGGAGGATGACAACATCATGAGGTCTCTACAGCTGTTCCAAAATGTCATGTAACGT
AGGACACTGGCCATCCTGCTCTCAGAGACACTGACAAACACCTCAATGCCCTGATCTGCCCTTGTTCCAGTTTTACACAT
CAACTCTCGGGACAGAAATACCTTTTACACTTTGGAAGAATTCTCTGCTGAAGACTTTCTACAAAACCTGGCACCGAGTG
GCTCAGTCTCTGATTGCCAACTCTTCTCCCTCCTCCTCTTGAGAGGGACGAGCTGAAATCCGAAGTTTGTGTTTGAAGC
ATGCCCATCTCTCCATGCTGCTGCTGCCCTGTGGAAGGCCCTCTGCTTGAGCTTAAACAGTAGTGCACAGTTTTCTGCG
TATACAGATCCCCAACTCACTGCCTCTAAGTCAGGCAGACCTGATCAATCTGAACCAAATGTGCACCATCCTCCGATGG
CCTCCCAAGCCAATGTGCCTGCTTCTTCTCCTCTGGTGGGAAGAAAGAACGCTCTACAGAGCACTTAGAGCTTACCATGA
AAATACTGGGAGAGGCAGCACCTAACACATGTAGAATAGGACTGAATTATTAAGCATGGTGGTATCAGATGATGCAACA
GCCCATGTCAATTTTTTTTTTCCAGAGGTAGGGACTAATAATTCTCCACACTAGCACCTACGATCATAGAACAAGTCTTTT
AACACATCCAGGAGGGAAACCGCTGCCAGTGGTCTATCCCTTCTCTCCATCCCTGCTCAAGCCCAGCACTGCATGTCT
CTCCCGGAAGGTCCAGAATGCCTGTGAAATGCTGTAACTTTTATACCTGTTATAATCAATAAACAGAACTATTTTCGTAC
AAAAAAAAAAAAAAAA

Fig. 2

RAT 1vN (r1vN) PROTEIN

MLTQGESEGLQTLGIVVLCSSLKLLHYLGLIDLSDDKIEDDLEMTMVCHRPEGLEQLEAQTNFTKRELQVLYRGFKNEC
PSGVVNEETFKQIYAQFFPHGDASTYAHYLFNAFDTTQTGSVKFEDFVTALSILLRGTVHEKLRWTFNLYDINKDGYINK
EEMMDIVKAIYDMMGKYTYPVLKEDTPRQHVDVFFQKMDKNKDGIVTLDEFLESCQEDDNIMRSLQLFQNVN

Fig. 2 Continued

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004260" 95294960

MOUSE 1V (CD:477-1127)

CGGCCCCCTGAGATCCAGCCCGAGCGCGGGGCGGAGCGGCCGGGTGGCAGCAGGGGCGGGCGGGCGGAGCGCAGCTCCCG
 CACCGCACGCGGCGCGGGCTCGGCAGCCTCGGCCGTGCGGGCACGCCGGCCCCGTGTCCAACATCAGGCAGGCTTTGGGG
 CTCGGGGCTCGGGCCTCGGAGAAGCCAGTGGCCCGGCTGGGTGCCCCGACCGGGGGGCGCCTGTCAAGGCTCCCGCGAGC
 CTCTGGCCCTGGGAGTCAGTGCATGTGCCTGGCTGAAGAAGGCAGCAGCCACGAGCTCCAGGCGCCCCGGCCCCACGTTT
 TCTGAATACCAAGCTGCAGGCGAGCTGCTCGGGGCTTTTTTGTCTTCTCGCTTTTCTCTCTCTCCAATTCAAAGTGGGCA
 ATCCACACCGATTTCTTTTCAGGGGAGGGAAGAGACAGGGCCTGGGGTCCCAAGACGCACACAAGTCTTCGCTGCCATGG
 GGGCCGTCATGGGCACCTTCTCCTCCCTGCAGACCAAACAAAGGCGACCTCTAAAGACAAGATTGAGGATGAGCTAGAG
 ATGACCATGGTTTGCCACCGGCCTGAGGGACTGGAGCAGCTTGAGGCACAGACGAACTTCACCAAGAGAGAACTGCAAGT
 CTTGTACCGGGGATTCAAAAACGAGTGCCCTAGCGGTGTGGTCAATGAAGAAACATTCAAGCAGATCTACGCTCAGTTTT
 TCCCTCACGGAGATGCCAGCACATATGCACATTACCTCTTCAATGCCTTCGACACCACCCAGACAGGCTCTGTAAAGTTC
 GAGGACTTTGTGACTGCTCTGTGATTTTACTGAGAGGGACAGTCCATGAAAACTAAGGTGGACGTTTAATTTGTATGA
 CATCAATAAAGACGGCTACATAAACAAAGAGGAGATGATGGACATAGTCAAAGCCATCTATGACATGATGGGAAATACA
 CCTATCCTGTGCTCAAAGAGGACACTCCCAGGCAGCATGTGGATGTCTTCTTCCAGAAAATGGATAAAAATAAAGATGGC
 ATTGTAACGTTAGATGAATTTCTTGAATCATGTGAGGAGATGACAACATCATGAGATCTCTACAGCTGTTCCAAAATGT
 CATGTAACAGGACACTGGCCATTCTGCTCTCAGAGACACTGACAAACACCTTAATGCCCTGATCTGCCCTTGTTCCAA
 TTTTACACACCAACTCTTGGGACAGAAATACCTTTTACACTTTGGAAGAATTCTCTGCTGAAGACTTTCTACAAAACCTG
 GCACCACGTGGCTCTGTCTCTGAGGGACGAGCGGAGATCCGACTTTGTTTTGGAAGCATGCCATCTCTTCATGCTGCTG
 CCCTGTGGAAGGCCCTCTGCTTGAGCTTAATCAATAGTGCACAGTTTTATGCTTACACATATCCCCAACTCACTGCCTC
 CAAGTCAGGCAGACTCTGATGAATCTGAGCCAAATGTGCACCATCCTCCGATGGCCTCCCAAGCCAATGTGCCTGCTTCT
 CTTCTCTGGTGGGAAGAAAGAGTGTTCTACGGAACAATTAGAGCTTACCATGAAAATATTGGGAGAGGCAGCACCTAAC
 ACATGTAGAATAGGACTGAATTATTAAGCATGGTGATATCAGATGATGCAAATTGCCCATGTCAATTTTTTTCAAAGGTAG
 GGACAAATGATTCTCCACACTAGCACCTGTGGTCATAGAGCAAGTCTCTTAACATGCCCAGAAGGGGAACCACTGTCCA
 GTGGTCTATCCCTCCTCTCCATCCCTGCTCAAACCCAGCACTGCATGTCCCTCCAAGAAGGTCCAGAATGCCTGCGAAA
 CGCTGTACTTTTATACCCTGTTCTAATCAATAAACAGAACTATTTTCGTAAAAAAAAAAAAAAAAAAAAA

MOUSE 1V PROTEIN

MGAVMGTFFSSLQTKQRRPSKDKIEDELEMTMVCHRPEGLEQLEAQTNFTKRELQVLYRGFKNECPSGVVNEETFQKIYAQ
 FFPHGDASTYAHYLFNAFDTTQTGSKVFEDFVTALSILLRGTVHEKLRTWTFNLYDINKDGYINKEEMMDIVKAIYDMMGK
 VTYPVLKEDTPRQHVDVFFQKMDKNKDGIVTLDEFLESCQEDDNIMRSLQLFQNVN

Fig. 3

RAT 1VL DNA (CD:31-714)

GTCCCAAGTCGCACACAAGTCTTCGCTGCCATGGGGGCCGTCATGGGTACCTTCTCGTCCCTGCAGACCAAACAAAGGCG
 ACCCTCTAAAGACATCGCCTGGTGGTATTACCAGTATCAGAGAGACAAGATCGAGGATGATCTGGAGATGACCATGGTTT
 GCCATCGGCCTGAGGGACTGGAGCAGCTTGAGGCACAGACGAACTTCACCAAGAGAGAAGTGAAGTCCCTTACCGGGGA
 TTCAAAAACGAGTGCCCCAGTGGTGTGGTTAACGAAGAGACATTCAAGCAGATCTACGCTCAGTTTTTCCCTCATGGAGA
 TGCCAGCACATACGCACATTACCTCTTCAATGCCTTCGACACCACCCAGACAGGCTCTGTAAAGTTCGAGGACTTTGTGA
 CTGCTCTGTGATTTTTACTGAGAGGAACGGTCCATGAAAACTGAGGTGGACGTTTAATTTGTACGACATCAATAAGAC
 GGCTACATAAAACAAAGAGGAGATGATGGACATAGTGAAAGCCATCTATGACATGATGGGGAAATACACCTATCCTGTGCT
 CAAAGAGGACACTCCCAGGCAGCACGTGGACGTCTTCTTCCAGAAAATGGATAAAAAATAAGATGGCATTTGTAACGTTAG
 ACGAATTTCTCGAGTCCTGTGAGGAGGATGACAACATCATGAGGTCTCTACAGCTGTTCCAAAATGTCATGTAAGTGGG
 AACTGGCCATCCTGCTCTCAGAGACACTGACAAACACCTCAATGCCCTGATCTGCCCTTGTTCCAGTTTTACACATCAA
 CTCTCGGGACAGAAATACCTTTTACACTTTGGAAGAATTCTCTGCTGAAGACTTTCTACAAAACCTGGCACCGCGTGGCT
 CAGTCTCTGATTGCCAACTCTTCTCCCTCCTCCTCTTGAGAGGGACGAGCTGAAATCCGAAGTTTGTTTTGGAAGCATG
 CCCATCTCTCCATGCTGCTGCTGCCCTGTGGAAGGCCCCCTCTGCTTGAGCTTAAACAGTAGTGCACAGTTTTCTGCGTAT
 ACAGATCCCCAACTCACTGCCTCTAAGTCAGGCAGACCCTGATCAATCTGAACCAAATGTGCACCATCCTCCGATGGCCT
 CCCAAGCCAATGTGCCTGCTTCTCTTCTCTGGTGGGAAGAAAGAACGCTCTACAGAGCACTTAGAGCTTACCATGAAAA
 TACTGGGAGAGGCAGCACCTAACACATGTAGAATAGGACTGAATTATTAAGCATGGTGGTATCAGATGATGCAACAGCC
 CATGTCAATTTTTTTTCCAGAGGTAGGGACTAATAATTCTCCACACTAGCACCTACGATCATAGAACAAGTCTTTTAACA
 CATCCAGGAGGGAAACCGCTGCCAGTGGTCTATCCCTTCTCTCCATCCCTGCTCAAGCCCAGCACTGCATGTCTCTCC
 CGGAAGGTCCAGAATGCCTGTGAAATGCTGTAACCTTTTATACCCTGTTATAATCAATAAACAGAACTATTTTCGTACAAA
 AAAAAAAAAAAAAA

RAT 1VL PROTEIN

MGAVMGTFSSLQTKQRRPSKDIAWYYYQYQRDKIEDDLEMTMVCHRPEGLEQLEAQTNFTKRELQVLYRGFKNECPSGVV
 NEETFQKIYAQFFPHGDASTYAHYLFNAFDTTQTGSVKFEDFVTALSILLRGTVHEKLRWTFNLVDINKDGYINKEEMMD
 IVKAIYDMMGKYTYPVLKEDTPRQHVDFVFFQKMDKNKDGIVTLDEFLESCQEDDNIMRSLQLFQNVN

Fig. 4

MOUSE 1VL DNA (CD:77-760)

ATCCACACCGATTCTTTTCAGGGGAGGGAAGAGACAGGGCCTGGGGTCCCAAGACGCACACAAGTCTTCGCTGCCATGG
 GGGCCGTCATGGGCACTTTCTCCTCCCTGCAGACCAAACAAAGGCGACCCCTCTAAAGACATCGCCTGGTGGTATTACCAG
 TATCAGAGAGACAAGATTGAGGATGAGCTAGAGATGACCATGGTTTGCCACCGGCCTGAGGGACTGGAGCAGCTTGAGGC
 ACAGACGAAC TTCACCAAGAGAGAACTGCAAGTCTTGTACCGGGGATTCAAAAACGAGTGCCCTAGCGGTGTGGTCAATG
 AAGAAACATTCAAGCAGATCTACGCTCAGTTTTTCCCTCACGGAGATGCCAGCACATATGCACATTACCTCTTCAATGCC
 TTCGACACCACCCAGACAGGCTCTGTAAAGTTCGAGGACTTTGTGACTGCTCTGTTCGATTTTACTGAGAGGGACAGTCCA
 TGAAAAACTAAGGTGGACGTTTAATTTGTATGACATCAATAAAGACGGCTACATAAACAAGAGGAGATGATGGACATAG
 TCAAAGCCATCTATGACATGATGGGGAAATACACCTATCCTGTGCTCAAAGAGGACACTCCCAGGCAGCATGTGGATGTC
 TTCTTCCAGAAAATGGATAAAAAATAAGATGGCATTGTAACGTTAGATGAATTTCTTGAATCATGTCAGGAGGATGACAA
 CATCATGAGATCTCTACAGCTGTTCCAAAATGTCATGTAAGTGGAGACACTGGCCATTCTGCTCTCAGAGACACTGACAA
 ACACCTTAATGCCCTGATCTGCCCTTGTTCCAATTTTACACACCAACTCTTGGGACAGAAATACCTTTTACACTTTGGAA
 GAATTCTCTGCTGAAGACTTTCTACAAAACCTGGCACCACGTGGCTCTGTCTCTGAGGGACGAGCGGAGATCCGACTTTG
 TTTTGGAAGCATGCCCATCTCTTCATGCTGCTGCCCTGTGGAAGGCCCTCTGCTTGAGCTTAATCAATAGTGCACAGTT
 TTATGCTTACACATATCCCCAACTCACTGCCTCCAAGTCAGGCAGACTCTGATGAATCTGAGCCAAATGTGCACCATCCT
 CCGATGGCCTCCCAAGCCAATGTGCCTGCTTCTCTTCTCTGGTGGGAAGAAAGAGTGTTCTACGGAACAATTAGAGCTT
 ACCATGAAAATATTGGGAGAGGCAGCACCTAACACATGTAGAATAGGACTGAATTATTAAGCATGGTGATATCAGATGAT
 GCAAATTGCCCATGTCAATTTTTTTCAAAGGTAGGGACAAATGATTCTCCACACTAGCACCTGTGGTCATAGAGCAAGTC
 TCTTAACATGCCCAGAAGGGGAACCACTGTCCAGTGGTCTATCCCTCCTCTCCATCCCCTGCTCAAACCCAGCACTGCAT
 GTCCCTCCAAGAAGGTCCAGAATGCCTGCGAAACGCTGTACTTTTATACCCTGTTCTAATCAATAAACAGAACTATTTTCG
 TACAAAAAAAAAAAAAAAAAAAA

MOUSE 1VL PROTEIN

MGAVMGTFFSSLQTKQRRPSKDIAWWYYQYQRDKIEDELEMTMVCHRPEGLEQLEAQTNFTKRELQVLYRGFKNECPSGVV
 NEETFKQIYAQFFPHGDASTYAHYLFNAFDTTQTGSVKFEDFVTALSILLRGTVHEKLRWTFNLYDINKDGYINKEEMD
 IVKAIYDMMGKYTYPVLKEDTPRQHVDVFFQKMDKNKDGIIVTLDEFLESCQEDDNIMRSLQLFQNMV

Fig. 5

RAT 1VN DNA (FIRST-PASS, PARTIAL; CD: 345-955)

GTCCGGGCACACAACCCCTGGATTCTTCGGAGAATATGCCGTGACGGTGTTGCCAATTATTAGTTCTCTTGGCTAGCAGA
TGTTTAGGGACTGGTTAAGCCTTTGGAGAAATTACCTTAGGAAAACGGGGAAATAAAAGCAAAGATTACCATGAATTGCA
AGATTACCTAGCAATTGCAAGGTAGGAGGAGAGAGGTGGAGGGCGGAGTAGACAGGAGGGAGGGAGAAAGTGAGAGGAAG
CTAGGCTGGTGGAAATAACCCTGCACTTGGAAACAGCGGCAAAGAAGCGCGATTTTCCAGCTTTAAATGCCTGCCCCGCTT
CTGCTTGCTTACCCGGGAACGGAGATGTTGACCCAGGGCGAGTCTGAAGGGCTCCAGACCTTGGGGATAGTAGTGGTCCCT
GTGTTCCCTCTCTGAACTACTGCACTACCTCGGGCTGATTGACTTGTTCGGATGACAAGATCGAGGATGATCTGGAGATGA
CCATGGTTTGGCATCGGCCTGAGGGACTGGAGCAGCTTGAGGCACAGACGAACTTCACCAAGAGAGAACTGCAAGTCCTT
TACCGGGGATTCAAAAACGAGTGCCCCAGTGGTGTGGTTAACGAAGAGACATTCAAGCNGATCTACGCTCAGTTTTTCCC
TCATGGAGATGCCAGCACATACGCACATTACCTCTTCAATGCCTTCGACACCACCCAGACAGGCTCTGTAAAGTTCGAGG
ACTTTGTGACTGCTCTGTTCGATTTTACTGAGAGGAACGGTCCATGAAAACTGAAGTGGACGTTTAATTTGTACGACATC
AATAAAGACGGCTACATAAACAAAGAGGAGATGATGGACATAGTGAAAGCCATCTATGACATGATGGGGAAATACACCTA
TCTTGTGCTCAAAGAGGACACTTCCAGGCAGCACGTGGACGTCTTCTTCCAGAAAATGGATAAAAATAAGATGG

RAT 1VN PROTEIN (PARTIAL)

MLTQGESEGLQTLGIVVVLCSLKLHLGLIDLSDDKIEDDLEMTMVCHRPEGLEQLEAQTNFTKRELQVLYRGFKNEC
PSGVVNEETFKKXIYAQFFPHGDASTYAHYLFNAFDTTQTGSVKFEDFVTALSILLRGTVEKCLKWTFNLYDINKDGYINK
EEMMDIVKAIYDMMGKYTYLVLKEDTSRQHVDFVFQKMDKNKD

Fig. 6

HUMAN 9QL DNA (CD:207-1019)

CTCACCTGCTGCCTAGTGTTCCTCTCCTGCTCCAGGACCTCCGGGTAGACCTCAGACCCCGGGCCCATTTCCAGACTCA
 GCCTCAGCCCGGACTTCCCCAGCCCCGACAGCACAGTAGGCCGCCAGGGGGCGCCGTGTGAGCGCCCTATCCCGGCCACC
 CGGGCCCCCTCCCACGGCCCCGGGCGGGAGCGGGGGCGCCGGGGGCCATGCGGGGCCAGGGCCGCAAGGAGAGTTTGTCCG
 ATTCCCCGAGACCTGGACGGCTCCTACGACCAGCTCACGGGCCACCCTCCAGGGCCCACTAAAAAGCGCTGAAGCAGCGA
 TTCCTCAAGCTGCTGCCGTGCTGCGGGCCCCAAGCCCTGCCCTCAGTCAGTGAAACATTAGCCGCCCCAGCCTCCCTCCG
 CCCCCACAGACCCCGCCTGCTGGACCCAGACAGCGTGGACGATGAATTTGAATTGTCCACCGTGTGTACCGGCCCTGAGG
 GTCTGGAGCAGCTGCAGGAGCAAACCAAATTCACGCGCAAGGAGTTGCAGGTCTGTACCGGGGCTTCAAGAACGAATGT
 CCCAGCGGAATTGTCAATGAGGAGAACTTCAAGCAGATTTACTCCCAGTTCTTTCTCAAGGAGACTCCAGCACCTATGC
 CACTTTTCTCTTCAATGCCTTTGACACCAACCATGATGGCTCGGTGAGTTTGTAGGACTTTGTGGCTGGTTTGTCCGTGA
 TTCTTCGGGGAAGTGTAGATGACAGGCTTAATTGGGCCTTCAACCTGTATGACCTTAACAAGGACGGCTGCATCACCAAG
 GAGGAAATGCTTGACATCATGAAGTCCATCTATGACATGATGGGCAAGTACACGTACCCTGCACTCCGGGAGGAGGCCCC
 AAGGGAACACGTGGAGAGCTTCTTCCAGAAGATGGACAGAAACAAGGATGGTGTGGTGACCATTGAGGAATTCATTGAGT
 CTTGTCAAAAGGATGAGAACATCATGAGGTCCATGCAGCTCTTTGACAATGTCATCTAGCCCCCAGGAGAGGGGGTCAGT
 GTTTCCTGGGGGACCATGCTCTAACCCTAGTCCAGGCGGACCTCACCCCTTCTCTTCCCAGGTCTATCCTCATCTACGC
 CTCCCTGGGGGCTGGAGGGATCCAAGAGCTTGGGGATTGAGTAGTCCAGATCTCTGGAGCTGAAGGGGCCAGAGAGTGGG
 CAGAGTGCATCTCGGGGGGTGTTCCCAACTCCCACCAGCTCTCACCCCTTCTCTGCTGACACCCAGTGTGAGAGTGCC
 CCTCCTGTAGGAATTGAGCGGTTCCCCACCTCCTACCTACTCTAGAAACACACTAGAGCGATGTCTCCTGCTATGGTGC
 TTCCCCCATCCCTGACCTCATAAACATTTCCCCTAAGACTCCCCTCTCAGAGAGAAATGCTCCATTCTTGGCACTGGCTGG
 CTTCTCAGACCAGCCATTGAGAGCCCTGTGGGAGGGGGACAAGAATGTATAGGGAGAAATCTTGGGCCTGAGTCAATGGA
 TAGGTCCTAGGAGGTGGGTGGGGTTGAGAATAGAAGGGCCTGGACAGATTATGATTGCTCAGGCATACCAGGTATAGCT
 CCAAGTTCCACAGGTCCTGCTACCACAGGCCATCAAAATATAAGTTTCCAGGCTTTCAGAGAAGACCTTGTCTCCTTAGAAA
 TGCCCCAGAAATTTTCCACACCCTCCTCGGTATCCATGGAGAGCCTGGGGCCAGATATCTGGCTCATCTCTGGCATTGCT
 TCCTCTCCTTCTCCTGTCATGTGTTGGTGGTGGTTGTGGTGGGGGAATGTGGATGGGGGATGTCTGGCTGATGCCTGC
 CAAAATTTTCATCCCACCCTCCTTGCTTATCGTCCCTGTTTTGAGGGCTATGACTTGAGTTTTTGTTCCTCATGTTCTCTA
 TAGACTTGGGACCTTCTGAACTTGGGGCCTATCACTCCCCACAGTGGATGCCCTAGAAGGGAGAGGGAAGGAGGGAGGC
 AGGCATAGC

Fig. 7

HUMAN 9QL PROTEIN

MRGQGRKESLSDSRDLGSDYDQLTGHPPGPTKKALKQRFLKLLPCCGPQALPSVSETLAAPASLRPHRPRLDPDSVDDE
FELSTVCHRPEGLEQLQEQTKEFTRKELQVLYRGFKNECPGIVNEENFKQIYSQFFPQGDSSTYATFLFNAFDTNHDGSV
SFEDFVAGLSVILRGTVDDRLNWAFNLYDLNKDGCITKEEMLDIMKSIYDMMGKYTYPALREEAPREHVESFFQKMDRNK
DGVVTIEEFIESCQKDENIMRSMQLFDNVI

Fig. 7 Continued

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RAT 9QL DNA (PARTIAL;CD:2-775)

CCGAGATCTGGACGGCTCCTATGACCAGCTTACGGGCCACCCCTCCAGGGCCCAGTAAAAAGCCCTGAAGCAGCGTTTCC
TCAAGCTGCTGCCGTGCTGCGGGCCCCAAGCCCTGCCCTCAGTCAGTGAAACATTAGCTGCCCCAGCCTCCCTCCGCCCC
CACAGACCCCGCCCGCTGGACCCAGACAGCGTAGAGGATGAGTTTGAATTATCCACGGTGTGTCACCGACCTGAGGGCCT
GGAACAACCTCCAGGAACAGACCAAGTTCACACGCAGAGAGCTGCAGGTCCTGTACCGAGGCTTCAAGAACGAATGCCCCA
GTGGGATTGTCAACGAGGAGAACCTCAAGCAGATTTATTCTCAGTTCTTTCCCCAAGGAGACTCCAGCAACTATGCTACT
TTTTCTTCAATGCCTTTGACACCAACCACGATGGCTCTGTCAGTTTTGAGGACTTTGTGGCTGGTTTGTGCGTGATTCT
TCGGGGGACCATAGATGATAGACTGAGCTGGGCTTTCAACTTATATGACCTCAACAAGGACGGCTGTATCACAAAGGAGG
AAATGCTTGACATTATGAAGTCCATCTATGACATGATGGGCAAGTACACATACCCTGCCCTCCGGGAGGAGGCCCAAGA
GAACACGTGGAGAGCTTCTTCCAGAAGATGGACAGGAACAAGGACGGCGTGGTGACCATCGAGGAATTCATCGAGTCTTG
TCAACAGGACGAGAACATCATGAGGTCCATGCAGCTCTTTGATAATGTCATCTAGCTCCCCAGGGAGAGGGGTTAGTGTG
TCCTAGGGTGACCAGGCTGTAGTCCTAGTCCAGACGAACCTAACCCCTCTCTCTCCAGGCCTGTCCTCATCTTACCTGTAC
CCTGGGGGCTGTAGGGATTCAATATCCTGGGGCTTCAGTAGTCCAGATCCCTGAGCTAAGTCACAAAAGTAGGCAAGAGT
AGGCAAGCTAAATCTGGGGGCTTCCCAACCCCGACAGCTCTCACCCCTTCTCAACTGATACCTAGTGCTGAGGACACCC
CTGGTGTAGGGACCAAGTGGTTCTCCACCTTCTAGTCCACTCTAGAAACCACATTAGACAGAAGGTCTCCTGCTATGGT
GCTTTCCCATCCCTAATCTCTTAGATTTTCTCAAGACTCCCTTCTCAGAGAACACGCTCTGTCCATGTCCCAGCTGG
GGACATGGACAGAGCGTGTTCTCTAGTTCTAGATCGCGAGCGGCCGC

RAT 9QL PROTEIN (PARTIAL)

RDLDGSYDQLTGHPGPSKKALKQRFLLPCCGPQALPSVSETLAAPASLRPHRPRPLDPDSVEDEFELSTVCHRPEGL
EQLQEQTKFTRRELQVLYRGFKNECPSGIVNEENFKQIYSQFFPQGDSSNYATFLFNAFDTNHDGSVSFEDFVAGLSVIL
RGTIDDRLSWAFNLYDLNKDGCITKEEMLDIMKSIYDMMGKYTPALREEAPREHVESFFQKMDRNDKGVVTIEEFIESC
QQDENIMRSMQLFDNVI

Fig. 8

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MOUSE 9QL DNA (CD:181-993)

CGGGACTCTGAGGTGGGCCCTAAAATCCAGCGCTCCCCAGAGAAAAGCCTTGCCAGCCCCCTACTCCCGGCCCCCAGCCCC
 AGCAGGTCGCTGCGCCGCCAGGGGGCACTGTGTGAGCGCCCTATCCTGGCCACCCGGCGCCCCCTCCACGGCCCCAGGCG
 GGAGCGGGGCGCCGGGGGCCATGCGGGGCCAAGGCCGAAAGGAGAGTTTGTCCGAATCCCAGATTGACGGCTCCTAT
 GACCAGCTTACGGGGCCACCCTCCAGGGCCCAGTAAAAAGCCCTGAAGCAGCGTTTCCTCAAGCTGCTGCCGTGCTGCGG
 GCCCCAAGCCCTGCCCTCAGTCAGTGAAACATTAGCTGCCCCAGCCTCCCTCCGCCCCACAGACCCCGCCCGCTGGACC
 CAGACAGCGTGGAGGATGAGTTTGAACATACACGGTGTGCCACCGGCCTGAGGGTCTGGAACAACCCAGGAACAAACC
 AAGTTCACACGCAGAGAGTTGCAGGTCTGTACAGAGGCTTCAAGAACGAATGTCCAGCGGAATTGTCAACGAGGAGAA
 CTTCAAGCAAATTTATTCTCAGTTCTTTCCCAAGGAGACTCCAGCAACTACGCTACTTTTCTCTTCAATGCCTTTGACA
 CCAACCATGATGGCTCTGTGAGTTTGTGAGGACTTTGTGGCTGGTTTGTGAGTGATTCTTCGGGGAACCATAGATGATAGA
 CTGAACTGGGCTTTCAACTTATATGACCTCAACAAGGATGGCTGTATCACGAAGGAGGAAATGCTCGACATCATGAAGTC
 CATCTATGACATGATGGGCAAGTACACCTACCCTGCCCTCCGGGAGGAGGCCCCGAGGGAACACGTGGAGAGCTTCTTCC
 AGAAGATGGACAGAAACAAGGACGGCGTGGTGACCATTGAGGAATTCATTGAGTCTTGTCACAGGACGAGAACATCATG
 AGGTCCATGCAACTCTTTGATAATGTCTAGCTCCCCAGGGAGAGGGGTTAGTGTGTCCCAGGGTAACCATGCTGTAG
 CCCTAGTCCAGGCAAACTAACCTCCTCTCCCGGGTCTGTCTCATCTACCTGTACCCTGGGGGCTGTAGGGATTCA
 ACATCCTGGCGCTTCAGTAGTCCAGATCCCTGAGCTAAGTGGCGAGAGTAGGCAAGCTAAGTCTTTGGAGGGTGGGTGGG
 GCGCGCAGATTCCCAACCCCCGACGACTCTCACCCTTTCTCGACTGATACCCAGTGCTGAGGCTACCCCTGGTGTCCG
 GAACGACCAAAGTGGTTCTCTGCCTCCCCAGCCCACTCTAGAGACCCACACTAGACGGGAATATCTCCTGCTATGGTGC
 TTCCCCATCCCTGACCGCAGATTTTCTCCTAAGACTCCCTTCTCAGAGAATATGCTTTTGTCCCTTGTCCCTGGCTGGC
 TTTTCAGCCTAGCCTTTGAGGACCCTGTGGGAGGGGAGAATAAGAAAGCAGACAAAATCTTGGCCCTGAGCCAGTGGTTA
 GGTCTTAGGAATCAGGCTGGAGTGAGACCAGAAAGCCTGGGCAGGCTATGAGAGCCCCAGGTTGGCTTGTACCGCCAG
 GTTCCACAGGGCTGCTGCTCTGGGTGAGCAGAGTATGAGTTTCCAGACTTTCCAGAAGGCCTTATGTCCTTAGCAATGTC
 CCAGAAATTCACCATACTTCTCAGTGTCTTAGGATCCAGATGTCCGGTCCATCCCTGAAACCTCTCCCTCCTCCTTGC
 TCCTATGGTGGGAGTGGTGGCCAGGGGACGATGAGTGAGCCGGTGTCTGGATGATGCCTGTCAAGGTCCCACCTACCC
 CCGGCTGTCAAGCCGTTCTGGTGACCCTGTTTGATTCTCCATGACCCCTGTCTAGATGTAGAGGTGTGGAGTGAGTCTAG
 TGGCAGCCTTAGGGGAATGGGAAGAAGAGAGGGGCACTCCATCTGAACCCAGTGTGGGGGCATCCATTCCAATCTTTGC
 CTGGCTCCCCACAATGCCCTAGGATCCTCTAGGGTCCCCACCCCACTCTTTAGTCTACCCAGAGATGCTCCAGAGCTCA
 CCTAGAGGGCAGGGACCATAGGATCCAGGTCCAACCTGTATCAGCATCCGGCCATGCTGCTGCTGCTTATTAATAAAC
 TGCTTGTGCTTCAGCGCCCCCTCCAGTCAGCCAGGGTCTGAGGGGAAGGCCCCCACTTTCCCGCCTCCTGTGAGACATT
 GTTGACTGCTTTGCATTTTGGGCTCTTCTACCTATATTTTGTATAATAAGAAAGACACCAGATCCAATAAAACACATGGC
 TATGCACAAAAA

MOUSE 9QL PROTEIN

MRGQGRKESLSERDLGSDYDQLTGHPGPSKKALKQRFLLPCCGPQALPSVSETLAAPASLRPHRPRPLDPDSVEDE
 FELSTVCHRPEGLEQLQEQTKFTRRELQVLYRGFKNECPSGIVNEENFKQIYSQFFPQGDSSNYATFLNADFDTNHGDSV
 SFEDFVAGLSVILRGITIDRLNFAFNLYDLNKDGCITKEEMLDIMKSIYDMMGKYTPALREEAPREHVESFFQKMDRKN
 DGVVTIEEFIESCQQDENIMRSMQLFDNVI

Fig. 9

HUMAN 9QM DNA (CD:207-965)

CTCACCTGCTGCCTAGTGTTCCCTCTCCTGCTCCAGGACCTCCGGGTAGACCTCAGACCCCGGGCCCATTTCCAGACTCA
 GCCTCAGCCCGGACTTCCCCAGCCCCGACAGCACAGTAGGCCGCCAGGGGGCGCCGTGTGAGCGCCCTATCCCGGCCACC
 CGGCGCCCCCTCCACGGCCCCGGGCGGGAGCGGGGCGCCGGGGGCCATGCGGGGCCAGGGCCGAAGGAGAGTTTGTCCG
 ATTCCCCGAGACCTGGACGGCTCCTACGACCAGCTCACGGGCCACCTTCCAGGGCCCACTAAAAAGCGCTGAAGCAGCGA
 TTCTTCAAGCTGCTGCCGTGCTGCGGGCCCCAAGCCCTGCCCTCAGTCAGTGAACACAGCGTGGACGATGAATTTGAATT
 GTCCACCGTGTGTACCCGGCTGAGGGTCTGGAGCAGCTGCAGGAGCAAACCAAATTCACGCGCAAGGAGTTGCAGGTCC
 TGTACCGGGGCTTCAAGAACGAATGTCCAGCGGAATTGTCAATGAGGAGAACTTCAAGCAGATTTACTCCCAGTTCTTT
 CCTCAAGGAGACTCCAGCACCTATGCCACTTTTCTCTTCAATGCCTTTGACACCAACCATGATGGCTCGGTTCAGTTTTGA
 GGACTTTGTGGCTGGTTTGTCCGTGATTCTTCGGGGAAGTGTAGATGACAGGCTTAATTGGGCCTTCAACCTGTATGACC
 TTAACAAGGACGGCTGCATCACCAAGGAGGAAATGCTTGACATCATGAAGTCCATCTATGACATGATGGGCAAGTACACG
 TACCCTGCACTCCGGGAGGAGGCCCCAAGGGAACACGTGGAGAGCTTCTTCCAGAAGATGGACAGAAACAAGGATGGTGT
 GGTGACCATTGAGGAATTCATTGAGTCTTGTCAAAAGGATGAGAACATCATGAGGTCCATGCAGCTCTTTGACAATGTCA
 TCTAGCCCCCAGGAGAGGGGGTCAGTGTTCCTGGGGGGACCATGCTCTAACCTAGTCCAGGCGGACCTCACCTTCTC
 TTCCCAGGTCTATCCTCATCCTACGCCCTCCCTGGGGGCTGGAGGGATCCAAGAGCTTGGGGATTCAGTAGTCCAGATCTC
 TGGAGCTGAAGGGGCCAGAGAGTGGGCAGAGTGCATCTCGGGGGTGTTCCTCAACTCCCACCAGCTCTCACCCCTTCTC
 GCCTGACACCCAGTGTTGAGAGTGCCCCCTCCTGTAGGAATTGAGCGGTTCCCCACCTCCTACCTACTCTAGAAAACAC
 TAGAGCGATGTCTCCTGTATGGTGCTTCCCCATCCCTGACCTCATAAACATTTCCCTAAGACTCCCCCTCAGAGAG
 AATGCTCCATTCTTGGCACTGGCTGGCTTCTCAGACCAGCCATTGAGAGCCCTGTGGGAGGGGGACAAGAATGTATAGGG
 AGAAATCTTGGGCCTGAGTCAATGGATAGGTCTTAGGAGGTGGGTGGGGTTGAGAATAGAAGGGCCTGGACAGATTATGA
 TTGCTCAGGCATACCAGGTTATAGCTCCAAGTTCCACAGGTCTGCTACCACAGGCCATCAAAATATAAGTTTCCAGGCTT
 TGCAGAAGACCTTGTCTCCTTAGAAATGCCCCAGAAATTTTCCACACCCCTCCTCGGTATCCATGGAGAGCCTGGGGCCAG
 ATATCTGGCTCATCTCTGGCATTGCTTCTCTCCTTCCCTTCCCTGCATGTGTTGGTGGTGGTTGTGGTGGGGGAATGTGGA
 TGGGGGATGTCTGGCTGATGCCTGCCAAATTTTCATCCACCCCTCCTTGTCTATCGTCCCTGTTTTGAGGGCTATGACT
 TGAGTTTTTGTTCCTCATGTTCTCTATAGACTTGGGACCTTCTTGAACCTTGGGGCCTATCACTCCCCACAGTGGATGCCT
 TAGAAGGGAGAGGGAAGGAGGGAGGCAGGCATAGC

Fig. 10

HUMAN 9QM PROTEIN

MRGQGRKESLSDSRDLGSDYDQLTGHPGPTKKALKQRFLKLLPCCGPQALPSVSENSVDDEFELSTVCHRPEGLEQLQE
QTKFTRKELQVLYRGFKNECPSGIVNEENFKQIYSQFFPQGDSSTYATFLFNAFDTNHDGSVSFEDFVAGLSVILRGTV
DRLNWAFNLYDLNKDGCITKEEMLDIMKSIYDMMGKYTYPALREEAPREHVESFFQKMDRNKDGVVTTIEEFIESCQK
DEN
IMRSMQLFDNVI

Fig. 10 Continued

096336-092700

RAT 9QM DNA (CD:214-972)

CTCACTTGCTGCCCCAAGGCTCCTGCTCCTGCCCCAGGACTCTGAGGTGGGCCCTAAAACCCAGCGCTCTCTAAAGAAAAG
 CCTTGCCAGCCCCCTACTCCCGCCCCCAACCCAGCAGGTCGCTGCGCCGCCAGGGGGCGCTGTGTGAGCGCCCTATTCT
 GGCCACCCGGCGCCCCCTCCACGGCCAGGCGGGAGCGGGGCGCCGGGGGCCATGCGGGGCCAAGGCAGAAAGGAGAGT
 TTGTCCGAATCCCGAGATCTGGACGGCTCCTATGACCAGCTTACGGGCCACCTCCAGGGCCCAGTAAAAAGCCCTGAA
 GCAGCGTTTCTCAAGCTGCTGCCGTGCTGCGGGCCCCAAGCCCTGCCCTCAGTCAGTGAAAACAGCGTAGAGGATGAGT
 TTGAATTATCCACGGTGTGTACCGACCTGAGGGCCTGGAACAACTCCAGGAACAGACCAAGTTCACACGCAGAGAGCTG
 CAGGTCTGTACCGAGGCTTCAAGAACGAATGCCCCAGTGGGATTGTCAACGAGGAGAACTTCAAGCAGATTTATTCTCA
 GTTCTTTCCCCAAGGAGACTCCAGCAACTATGCTACTTTTCTCTTCAATGCCTTTGACACCAACCACGATGGCTCTGTCA
 GTTTTGAGGACTTTGTGGCTGGTTTGTGCGGTGATTCTTCGGGGGACCATAGATGATAGACTGAGCTGGGCTTTCAACTTA
 TATGACCTCAACAAGGACGGCTGTATCACAAGGAGGAAATGCTTGACATTATGAAGTCCATCTATGACATGATGGGCAA
 GTACACATACCCTGCCCTCCGGGAGGAGGCCCCAAGAGAACACGTGGAGAGCTTCTTCAGAAGATGGACAGGAACAAGG
 ACGGCGTGGTGACCATCGAGGAATTCATCGAGTCTTGTCAACAGGACGAGAACATCATGAGGTCCATGCAGCTCTTTGAT
 AATGTCATCTAGCTCCCCAGGGAGAGGGGTTAGTGTGTCTTAGGGTGACCAGGCTGTAGTCCTAGTCCAGACGAACCTAA
 CCCTCTCTCTCCAGGCCTGTCTCATCTTACCTGTACCCTGGGGGCTGTAGGGATTCAATATCCTGGGGCTTCAGTAGTC
 CAGATCCCTGAGCTAAGTCACAAAAGTAGGCAAGAGTAGGCAAGCTAAATCTGGGGGCTTCCCAACCCCCGACAGCTCTC
 ACCCTTCTCAACTGATACCTAGTGCTGAGGACACCCCTGGTGTAGGGACCAAGTGGTTCTCCACCTTCTAGTCCCCTC
 TAGAAACCACATTAGACAGAAGGTCTCTGCTATGGTGCTTTCCCCATCCCTAATCTCTTAGATTTTCTCAAGACTCCC
 TTCTCAGAGAACACGCTCTGTCCATGTCCCCAGCTGGCTTCTCAGCCTAGCCTTTGAGGGCCCTGTGGGGAGGCGGGGAC
 AAGAAAGCAGAAAAGTCTTGGCCCCGAGCCAGTGGTTAGGTCTTAGGAATTGGCTGGAGTGGAGGCCAGAAAGCCTGGGC
 AGATGATGAGAGCCCAGCTGGGCTGTCACTGCAGGTTCCGGGGCTACAGCCCTGGGTGAGCAGAGTATGAGTTCCCAGA
 CTTTCCAGAAGGTCTTAGCAATGTCCAGAAATTCACCGTACACTTCTCAGTGTCTTAGGAGGGCCCGGATCCAGATG
 TCTGGTTCATCCCTGAATCCTCTCCCTCCTTCTTGCTCGTATGGTGGGAGTGGTGGCCAGGGGAAGATGAGTGGTGTCCC
 GGATGATGCCTGTCAAGGTCCCACCTCCCTCCGGCTGTTCTCATGACAGCTGTTTGGTTCTCCATGACCCCTATCTAGA
 TGTAGAGGCATGGAGTGTGTCAGGGATTTCGGAACCTTGAGTTTTACCACTCCTCCTAGTGGCTGCCTTAGGGGAATGGG
 AAGAACCAGTGTGGGGGACCCATTAGAATCTTTGCCCCGCTCCTCACAATGCCCTAGGGTCCCCAGGGTACCCGCTC
 CCTCTGTTTAGTCTACCCAGAGATGCTCCTGAGCTACCTAGAGGTTAGGGACGGTAGGCTCCAGGTCCAACCTCTCCAG
 GTCAGACCCCTGCCATGCTGCTGCTCCTCATTAACAAACCTGCTTGTCTCCTCCTGCGCCCCCTTCTCAGTCAGCCAGGGT
 CTGAGGGGAAGGGCCTCCCGTTTCCCCATCCGTGAGACATGGTTGACTGCTTTGCATTTTGGGCTCTTCTATCTATTTTG
 TAAAAATAAGACATCAGATCCAATAAAACACACGGCTATGCACAAAAAAAAAAAAAAAAAAAA

RAT 9QM PROTEIN

MRGQGRKESLSERDLGSDYDQLTGHPGPSKKALKQRFLKLLPCCGPQALPSVSENSVEDEFELSTVCHRPEGLEQLQE
 QTKFTRRELQVLYRGFKNECPSGIVNEENFKQIYSQFFPQGDSSNYATFLNAFDTNHDGSVSFEDFVAGLSVILRGITD
 DRLSWAFNLYDLNKDGCITKEMLDIMKSIYDMMGKYTYPALREEAPREHVESFFQKMDRNKDGVVITIEEFIESCQQDEN
 IMRSMQLFDNVI

Fig. 11

CTCACCTGCTGCCTAGTGTTCCTCTCCTGCTCCAGGACCTCCGGGTAGACCTCAGACCCCGGGCCCATTTCCAGACTCA
GCCTCAGCCCGGACTTCCCCAGCCCCGACAGCACAGTAGGCCGCCAGGGGGCGCCGTGTGAGCGCCCTATCCCGGCCACC
CGGCGCCCCCTCCACGGCCCGGGCGGGAGCGGGGCGCCGGGGGCCATGCGGGGCCAGGGCCGCAAGGAGAGTTTGTCCG
ATTTCCCGAGACCTGGACGGCTCCTACGACCAGCTCACGGACAGCGTGGACGATGAATTTGAATTGTCCACCGTGTGTAC
CGGCCCTGAGGGTCTGGAGCAGCTGCAGGAGCAAACCAAATTCACGCGCAAGGAGTTGCAGGTCTGTACCGGGGCTTCAA
GAACGAATGTCCAGCGGAATTGTCAATGAGGAGAACTTCAAGCAGATTTACTCCAGTTCTTTCTCTCAAGGAGACTCCA
GCACCTATGCCACTTTTCTCTTCAATGCCTTTGACACCAACCATGATGGCTCGGTCAGTTTTGAGGACTTTGTGGCTGGT
TTGTCCGTGATTCTTCGGGGAACGTAGATGACAGGCTTAATTGGGCCTTCAACCTGTATGACCTTAACAAGGACGGCTG
CATCACCAGGAGGAAATGCTTGACATCATGAAGTCCATCTATGACATGATGGGCAAGTACACGTACCCTGCACTCCGGG
AGGAGGCCCCAAGGGAACACGTGGAGAGCTTCTTCCAGAAGATGGACAGAAACAAGGATGGTGTGGTGACCATTGAGGAA
TTCATTGAGTCTTGTCAAAGGATGAGAACATCATGAGGTCCATGCAGCTCTTTGACAATGTATCTAGCCCCCAGGAGA
GGGGGTCAGTGTTTTCTTGGGGGGACCATGCTCTAACCCCTAGTCCAGGCGGACCTCACCCCTTCTCTTCCCAGGTCTATCCT
CATCTACGCCTCCCTGGGGGCTGGAGGGATCCAAGAGCTTGGGGATTCAGTAGTCCAGATCTCTGGAGCTGAAGGGGCC
AGAGAGTGGGCAGAGTGCATCTCGGGGGGTGTTCCCAACTCCCACCAGCTCTCACCCCTTCTTGCCTGACACCCAGTGT
TGAGAGTGCCCCCTCCTGTAGGAATTGAGCGGTTCCCCACCTCCTACCCCTACTCTAGAAACACACTAGAGCGATGTCTCCT
GCTATGGTGCCTTCCCCCATCCCTGACCTCATAAACATTTCCCTTAAGACTCCCTCTCAGAGAGAATGCTCCATTCTTGG
CACTGGCTGGCTTCTCAGACCAGCCATTGAGAGCCCTGTGGGAGGGGGACAAGAATGTATAGGGAGAAATCTTGGGCCTG
AGTCAATGGATAGGTCCTAGGAGGTGGGTGGGGTTGAGAATAGAAGGGCCTGGACAGATTATGATTGCTCAGGCATACCA
GGTTATAGCTCCAAGTTCACAGGTCTGCTACCACAGGCCATCAAAATATAAGTTTCCAGGCTTTGCAGAAGACCTTGTC
TCCTTAGAAATGCCCCAGAAATTTCCACACCCCTCCTCGGTATCCATGGAGAGCCTGGGGCCAGATATCTGGCTCATCTC
TGGCATTGCTTCTCTCCTTCTCCTGCATGTGTTGGTGGTGGTGTGTTGGTGGGGGAATGTGGATGGGGGATGTCTTGGC
TGATGCCTGCCAAAATTTTCATCCCACCCCTCCTTGCTTATCGTCCCTGTTTTGAGGGCTATGACTTGAGTTTTTGTTTCCC
ATGTTCTCTATAGACTTGGGACCTTCTGAACTTGGGGCCTATCACTCCCCACAGTGGATGCCTTAGAAGGGAGAGGGAA
GGAGGGAGGCAGGCATAGC

MONKEY 9QS DNA (CD:133-795)

CCCACGCGTCCGCCCACGCGTCCGCGGACGCGTGGGGTGCCTAGGCCGCCAGGGGGCGCCGTGTGAGCGCCCTATCCC
 GCCACCCGGCGCCCCCTCCACGGACCGGGCGGGAGCGGGGGCGCCGGGGGCCATGCGGGGCCAGGGCCGCAAGGAGAGTT
 TGTCCGATTCCCCGAGACCTGGACGGATCCTACGACCAGCTCACGGACAGCGTGGAGGATGAATTTGAATTGTCCACCGTG
 TGTACCCGGCCTGAGGGTCTGGAGCAGCTGCAGGAGCAAACCAAATTCACGCGCAAGGAGTTGCAGGTCTGTACCGGGG
 CTTCAAGAACGAATGTCCGAGCGGAATTGTCAATGAGGAGAATTCAAGCAAATTTACTCCCAGTTCCTTCTCAAGGAG
 ACTCCAGCACCTATGCCACTTTTCTCTTCAATGCCTTTGACACCAACCATGATGGCTCGGTGAGTTTGTAGGACTTTGTG
 GCTGGTTTGTCCGTGATTCTTCGGGGAACGTAGATGACAGGCTTAATTGGGCTTCAACTTGTATGACCTCAACAAGGA
 CGGCTGCATCACCAAGGAGGAAATGCTTGACATCATGAAGTCCATCTATGACATGATGGGCAAGTACACATACCCTGCAC
 TCCGGGAGGAGGCCCAAGGGAACATGTGGAGAATTCTTCCAGAAGATGGACAGAAACAAGGATGGCGTGGTGACCATT
 GAGGAATTCATTGAGTCTTGTCAAAAGGATGAGAACATCATGAGGTCCATGCAGCTCTTTGACAATGTATCTAGCCCC
 AGGAGAGGGGGTCAGTGTCTTCTGGGGGACCATGCTCTAACCTAGTCCAGGTGGACCTCACCTTCTCTTCCCAGGTC
 TATCCTTGTCTTAGGCCTCCCTGGGGCTGGAGGGATCCAAGAGCTTGGGGATTGAGTAGTCCAGATCTCTGGAGCTGAA
 GGGGCCAGAGAGTGGGCAGAGTGCATCTTGGGGGTGTTCCCAACTCCCACCAGCTTTCACCCGCTTCTGCTGACACC
 CAGTGTGAGAGTGCCCCCTCTGTAGGAACGTAGTGGTTCCCCACCTCCTACCCCCACTCTAGAAACACACTAGACAGAT
 GTCTCGTGCTATGGTGCTTCCCCCATCCCTGACTTCATAAACATTTCCCCTAAACTCCCTTCTCAGAGAGAATGCTCCA
 TTCTTGGCACTGGCTGGCTTCTCAGACCAGCCTTTGAGAGCCCTGTGGGAGGGGACAAGAATGTATAGGGGAGAAATCT
 TGGGCCTGAGTCAATGGATAGGTCTTAGGAGGTGGCTGGGGTTGAGAATAGAAAGGCTGGACACAATGTGATTGCTCAG
 GCATACCAAGTTATAGCTCCAAGTTCCACAGGTCTGCTACCACAGGCCATCAAAATATAAGTTTCCAGGCTTTGCAGAAG
 ACCTTGTCTCCTTGGAATGCCCCAGATATTTCCATACCTCCTCGATATCCATGGAGAGCCTGGGGCTAGATATCTGG
 CATATCCCTGGCATTGCTTCTCTCCTTCTTCTGCTGCTGCTGGTGGTGGTGTGGCAGGGGAATGTGGATAGGAGAT
 GTCCTGGCAGATGCCTGCCAAAGTTTCATCCACCTCCTGCTCATCGCCCTGTTTTGAGGGCTGTGACTTGAGTTTT
 TGTTTTCCCATGTTCTCTATAGACTTGGGACCTTCTGAACTTGGGGCCTATCACTCCCCACAGTGGATGCCTTAGAAGGG
 AGAGGGAAGGAGGGAGGCAGGCATAGCATCTGAACCCAGTGTGGGGGCATTCACTAGGATCTTCAATCAACCCGGGCTCT
 CCCCACCCCCCAGATAACCTCCTCAGTTCCCTAGAGTCTCCTCTTGCTCTACTCAATCTACCCAGAGATGCCCCCTTAGC
 AACTCAGAGGGCAGGGACCATAGGACCCAGGTTCACCCCCATTGTCAGCACCCAGCCATGCTGCCATCCCTTAGCAC
 ACCTGCTCGTCCATTGAGCTTACCCTCCAGTCAGCCAGAATCTGAGGGGAGGGCCCCCAGAGAGCCCCCTTCCCCATC
 AGAAGACTGTTGACTGCTTTGCATTTTGGGCTCTCTATATATTTTGTAATAAGAACTATACCAGATCTAATAAAACA
 CAATGGCTATGCAAAAAAAAAAAAAAAAAAAAA

MONKEY 9QS PROTEIN

MRGQGRKESLSDSRDLGSDYDQLTDSVEDEFELSTVCHRPEGLEQLQEQTKEFTRKELQVLYRGFKNECPSGIVNEENFKQ
 IYSQFFPQDSSYATFLNADFDTNHDGVSFEDFVAGLSVILRGTVDDRNLNWFNLYDLNKDGCITKEMLDIMKSIYD
 MMGKYTPALREEAPREHVENFFQKMDRNDGVTIEEFIESCQKDENIMRSMQLFDNVI

Fig. 13

RAT 9QC DNA (CD:208-966)

TGCTGCCCCAAGGCTCCTGCTCCTGCCCCAGGACTCTGAGGTGGGCCCTAAAACCCAGCGCTCTCTAAAGAAAAGCCTTGC
 CAGCCCCTACTCCCGGCCCCCAACCCAGCAGGTCGCTGCGCCGCCAGGGGGCGCTGTGTGAGCGCCCTATTCTGGCCAC
 CCGGCGCCCCCTCCCACGGCCCAGGCGGGAGCGGGGCGCCGGGGGGCCATGCGGGGCCAAGGCAGAAAGGAGAGTTTGTCC
 GAATCCCAGATCTGGACGGCTCCTATGACCAGCTTACGGGCCACCCCTCCAGGGCCCAGTAAAAAGCCCTGAAGCAGCG
 TTTCTCAAGCTGCTGCCGTGCTGCGGGCCCCAAGCCCTGCCCTCAGTCAGTGAAAACAGCGTAGAGGATGAGTTTGAAT
 TATCCACGGTGTGTACCCGACCTGAGGGCCTGGAACAACCTCCAGGAACAGACCAAGTTCACACGCAGAGAGCTGCAGGTC
 CTGTACCGAGGCTTCAAGAACGAATGCCCCAGTGGGATTGTCAACGAGGAGAACTTCAAGCAGATTTATTCTCAGTTCTT
 TCCCCAAGGAGACTCCAGCAACTATGCTACTTTTCTCTTCAATGCCTTTGACACCAACCACGATGGCTCTGTGAGTTTGT
 AGGACTTTGTGGCTGGTTTGTGCGGTGATTCTTCGGGGGACCATAGATGATAGACTGAGCTGGGCTTTCAACTTATATGAC
 CTCAACAAGGACGGCTGTATCACAAGGAGGAAATGCTTGACATTATGAAGTCCATCTATGACATGATGGGCAAGTACAC
 ATACCCTGCCCTCCGGGAGGAGGCCCAAGAGAACACGTGGAGAGCTTCTTCCAGAAGATGGACAGGAACAAGGACGGCG
 TGGTGACCATCGAGGAATTCATCGAGTCTTGTCAACAGGACGAGAACATCATGAGGTCCATGCAGCTCTCACCCCTTCTC
 AACTGATACCTAGTGTGAGGACACCCCTGGTGTAGGACCAAGTGGTTCTCCACCTTCTAGTCCCCTCTAGAAACCAC
 ATTAGACAGAAGGTCTCTTGCTATGGTGCTTTCCCCATCCCTAATCTCTTAGATTTTCTCAAGACTCCCTTCTCAGAGA
 ACACGCTCTGTCCATGTCCCCAGCTGGCTTCTCAGCCTAGCCTTTGAGGGCCCTGTGGGGAGGCGGGGACAAGAAAGCAG
 AAAAGTCTTGGCCCCGAGCCAGTGGTTAGGTCTTAGGAATTGGCTGGAGTGGAGGCCAGAAAAGCCTGGGCAGATGATGAG
 AGCCCAGCTGGGCTGTCACTGCAGGTTCCGGGGCCTACAGCCCTGGGTGAGCAGAGTATGAGTTCCAGACTTTCCAGAA
 GGTCTTAGCAATGTCCAGAAATTCACCGTACACTTCTCAGTGTCTTAGGAGGGCCCCGGATCCAGATGTCTGGTTTAT
 CCCTGAATCCTCTCCCTCCTTCTTGCTCGTATGGTGGGAGTGGTGGCCAGGGGAAGATGAGTGGTGTCCCGGATGATGCC
 TGTCAAGGTCCCACCTCCCCCTCCGGCTGTTCTCATGACAGCTGTTTGGTTCTCCATGACCCCTATCTAGATGTAGAGGCA
 TGGAGTGAGTCAGGGATTTCCCGAACTTGAGTTTTACCACTCCTCCTAGTGGCTGCCTTAGGGGAATGGGAAGAACCAG
 TGTGGGGGCACCCATTAGAAATCTTTGCCCCGCTCCTCACAATGCCCTAGGGTCCCCTAGGGTACCCGCTCCCTCTGTTTA
 GTCTACCCAGAGATGCTCCTGAGCTCACCTAGAGGGTAGGGACGGTAGGCTCCAGGTCCAACCTCTCCAGGTCAGCACC
 TGCCATGCTGCTGCTCCTCATTAACAAACCTGCTTGTCTCCTCCTGCGCCCCCTTCTCAGTCAGCCAGGGTCTGAGGGGAA
 GGGCCTCCCGTTTCCCCATCCGTCAGACATGGTTGACTGCTTTGCATTTTGGGCTCTTCTATCTATTTTGTAAATAAGA
 CATCAGATCCAATAAAACACACGGCTATGCACAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

RAT 9QC PROTEIN

MRGQGRKESLSESRDLGSDYDQLTGHPPGPSKKALKQRFLLPCCGPQALPSVSENSVEDEFELSTVCHRPEGLEQLQE
 QTKFTRRELQVLYRGFKNECPSGIVNEENFKQIYSQFFPQGDSSNYATFLFNAFDTNHDSVSFEDFVAGLSVILRGTID
 DRLSWAFNLYDLNKDGCITKEEMLDIMKSIYDMMGKYTYPALREEAPREHVESFFQKMDRNDKGVVTTIEEFIESCQQDEN
 IMRSMQLSPLLN

Fig. 14

RAT 8T (9Q SPLICE VARAIANT) DNA (MAY NOT BE FULL LENGTH, CD: 1-678)

ATGAACCACTGCCCTCGCAGGTGCCGGAGCCCGTTGGGGCAGGCAGCTCGATCTCTCTACCAGTTGGTAACTGGGTCGCT
 GTCGCCAGACAGCGTAGAGGATGAGTTTGAATTATCCACGGTGTGTCACCGACCTGAGGGCCTGGAACAACTCCAGGAAC
 AGACCAAGTTCACACGCAGAGAGCTGCAGGTCTGTACCGAGGCTTCAAGAACGAATGCCCCAGTGGGATTGTCAACGAG
 GAGAACTTCAAGCAGATTTATTCTCAGTTCTTTCCCAAGGAGACTCCAGCAACTATGCTACTTTTCTCTTCAATGCCTT
 TGACACCAACCACGATGGCTCTGTCTAGTTTGTGAGGACTTTGTGGCTGGTTTGTCCGGTGATTCTTCGGGGGACCATAGATG
 ATAGACTGAGCTGGGCTTTCAACTTATATGACCTCAACAAGGACGGCTGTATCACAAGGAGGAAATGCTTGACATTATG
 AAGTCCATCTATGACATGATGGGCAAGTACACATACCCTGCCCTCCGGGAGGAGGCCCCAAGAGAACACGTGGAGAGCTT
 CTTCCAGAAGATGGACAGGAACAAGGACGGCGTGGTGACCATCGAGGAATTCATCGAGTCTTGTCAACAGGACGAGAACA
 TCATGAGGTCCATGCAGCTCTTTGATAATGTCATCTAGCTCCCCAGGGAGAGGGGTTAGTGTGTCTAGGGTGACCAGGC
 TGTAGTCCTAGTCCAGACGAACCTAACCTCTCTCTCCAGGCCTGTCTCATCTTACCTGTACCCTGGGGGCTGTAGGGA
 TTCAATATCTTGGGGCTTCAGTAGTCCAGATCCCTGAGCTAAGTCACAAAAGTAGGCAAGAGTAGGCAAGCTAAATCTGG
 GGGCTTCCCAACCCCGACAGCTCTCACCCCTTCTCAACTGATACCTAGTGCTGAGGACACCCCTGGTGTAGGGACCAAG
 TGGTCTCCACCTTCTAGTCCCCTCTAGAAACCACATTAGACAGAAGGTCTCTGCTATGGTGCTTTCCCATCCCTAA
 TCTCTTAGATTTTCTCAAGACTCCCTTCTCAGAGAACACGCTCTGTCCATGTCCCCAGCTGGCTTCTCAGCCTAGCCTT
 TGAGGGCCCTGTGGGGAGGCGGGGACAAGAAAGCAGAAAAGTCTTGGCCCCGAGCTAGTGGTTAGGTCCTAGGAATTGGC
 TGGAGTGGAGGCCAGAAAGCCTGGGCAGATGATGAGAGCCCAGCTGGGCTGTCACTGCAGGTTCCAGGGCCTACAGCCCT
 GGGTCAGCAGAGTATGAGTTCCAGACTTTCCAGAAGGTCTTAGCAATGTCCCAGAAATTCACCATACTTCTCAGTG
 TCCCGGATGATGCCTGTCAAGGTCCCACCTCCCTCCGGCTGTTTCTCATGACAGCTGTTTGGTCTCCATGACCCCTATC
 TAGATGTAGAGGCATGGAGTGAGTCAGGGATTCCCGAACTTGAGTTTACCCTCCTCCTAGTGGCTGCCCTTAGGGGAA
 TGGGAAGAACCCAGTGTGGGGGCACCCATTAGAATCTTTGCCCGGTTCCCTCACAATGCCCTAGGGTCCCTAGGGTACCC
 GCTCCCTCTGTTTAGTCTACCCAGAGATGCTCCTGAGCTCACCTAGAGGGTAGGGACGGTAGGCTCCAGGTCCAACCTCT
 CCAGGTGAGCACCCTGCCATGCTGCTGCTCCTCATTAACAAACCTGCTTGTCTCCTCCTGCGCCCTTCTCAGTCAGCCA
 GGGTCTGAGGGGAAGGGCCTCCCGTTTCCCATCCGTCAGACATGGTTGACTGCTTTGCATTTTGGGCTCTTCTATCTAT
 TTTGTAAATAAGACATCAGATCCAATAAAACACACGGCTATGCACAAAAAAAAAAAAAAAAAAAA

RAT 8T (9Q SPLICE VARAIANT) PROTEIN (MAY NOT BE FULL LENGTH)

MNHCPRRCRSPLGQAARSLYQLVTGSLSPDSVEDEFELSTVCHRPEGLEQLQEQTKEFTRRELQVLYRGFKNECPSGIVNE
 ENFKQIYSQFFPQGDSSNYATFLFNAFDTNHDGSVSFEDFVAGLSVILRGTIDDRLSWAFNLYDLNKDGCITKEMLDIM
 KSIYDMMGKYTYPALREEAPREHVESFFQKMDRNDGTVVTTIEEFIESCQQDENIMRSMQLFDNVI

Fig. 15

>human KChIP3 cds=1-7:
 ATGCAGCCGGCTAAGGAAGTGACAAAGGCGTCGGACGGCAGCCTCCTGGGGGACCTCGGGC
 ACACACCACTTAGCAAGAA
 GGAGGGTATCAAGTGGCAGAGGCCGAGGCTCAGCCGCCAGGCTTTGATGAGATGCTGCCTG
 GTCAAGTGGATCCTGTCCA
 GCACAGCCCCACAGGGCTCAGATAGCAGCGACAGTGAGCTGGAGCTGTCCACGGTGCGCCA
 CCAGCCAGAGGGGCTGGAC
 CAGCTGCAGGCCCAGACCAAGTTCACCAAGAAGGAGCTGCAGTCTCTCTACAGGGGCTTTA
 AGAATGAGTGTCCCACGGG
 CCTGGTGGACGAAGACACCTTCAAACATTTACGCGCAGTTCTTCCCTCAGGGAGATGCCA
 CCACCTATGCACACTTCC
 TCTTCAACGCCTTTGATGCGGACGGGAACGGGGCCATCCACTTTGAGGACTTTGTGGTTGGC
 CTCTCCATCCTGCTGCGG
 GGCACAGTCCACGAGAAGCTCAAGTGGGCCTTTAATCTCTACGACATTAACAAGGATGGCT
 ACATCACCAAAGAGGAGAT
 GCTGGCCATCATGAAGTCCATCTATGACATGATGGGCCGCCACACCTACCCCATCCTGCGGG
 AGGACGCGCCGGCGGAGC
 ACGTGGAGAGGTTCTTCGAGAAAAATGGACCGBAACAGGATGGGGTAGTGACCATTGAAGA
 GTTCCTGGAGGCCTGTCAG
 AAGGATGAGAACATCATGAGCTCCATGCAGCTGTTTGAGAATGTCATCTAGgacacgtccaaaggagt
 gcatggccacag
 ccacctccaccccccaagaaacctccatcctgccaggagcagcctccaagaaacttttaaaaaatagatttgcaaaaagtg
 aacagattgctacacacacacacacacacacacacacacacacacagccattcatctgggctggcagaggggac
 agagttcagggaggggctgagtctggctaggggcccaggtccaggagccccagccagcccttcccaggccagcgagggcgag
 gctgcctctgggtgagtggctgacagagcaggtctgcaggccaccagctgctggatgtcaccaagaaggggctcgagtgc
 cctgcaggggaggggtccaatctccggtgtgagcccacctcgtcccgttctccattctgctttcttgccacacagtgggc
 cggccccaggtccccctgggtctcctccccgtagccactctctgccactacctatgcttctagaaagccccctcacctcag
 gacccagagggaccagctggggggcaggggggagaggggtaatggaggccaagcctgcagctttctggaaattcttcc
 ctgggggtcccaggatccccctgctactccactgacctggaagagctgggtaccaggccaccactgtggggcaagcctga
 gtggtgagggggcactgggccccattctccctccatggcaggaaggcggggatttcaagtttagggattgggtcgtggt
 ggagaatctgagggcactctctgccagctccacaggggtgggatgagcctctccttgcccagtcctgggtcagtggaat
 gcagtgggtggggctgtacacacctccagcacagactgttccctccaaggtcctcttaggtcccgggaggaacgtggtt
 cagagactggcagccaggagccccggggcagagctcagaggagtctgggaaggggctgtccctcctcttctgtagtgc
 ccctcccagggccagcagcttaggctgagccccctctcctgaagcagtgctgcgcgtccctctgccttgcacaaaaagcac
 aagcattccttagcagctcaggcgagccctagtgggagcccagcacactgcttctcgaggccaggccctcctgctggc
 tgaggcttggggccagtagcccaaatatggtggccctggggaagaggccttgggggtctgctctgtgcctgggatcagtg
 gggcccaaaagcccagcccggctgaccaacattcaaaagcacaacccctggggactctgcttggctgtcccctccatctg
 gggatggagaatgccagcccaaagctggagccaatggtgagggctgagagggctgtggctgggtgggtcagcagaaacccc
 caggaggagagagatgctgctcccgcctgattggggcctcaccagaaaggaacccgggtcccaggccgcatggcccccca
 ggaacattcccacataatacattccatcacagccagcccagctccactcagggctggccccggggagtccccgtgtgcccc
 aagaggtagccccagggtgagcagggccctcagaggaaaggcagtatggcggaggccatggggggccccctcggcattcac
 acacagcctggcctcccctgcggagctgcatggacgcctgggtccagggtccagggtgactgggggcctctgcctccagg
 agggcatcagctttccctgggtcagggatcttctccctccccctcaccgctgcccagccctcccagctgggtgtcactctg
 cctctaaggccaaggcctcaggagagcatcaccaccacacccctgccggccttggccttggggccagactgggtgcacag
 cccaaccaggaggggtctgcctcccacgctgggacacagaccggccgcatgtctgcatggcagaagcgtctcccaggcc
 acggcctgggaggggtggttctgttctcagcatccactaatattcagtcctgtatattttaataaaataaacttgacaaa
 ggaaaaaaaaaaaaaaaaaattcctgcggccgcgttctcca

Fig.16

>human KChIP3
MQPAKEVTKASDGSLLGDLGHTPLSKKEGIKWQRPRLSRQALMRCCLVKWILSSTAPQGSDDSD
SELELSTVRHQPEGLD
QLQAQTKFTKKELQSLYRGFKNECPTGLVDEDTFKLIYAQFFPQGDATTYAHFLFNAFDADGNG
AIHFEDFVVGLSILLR
GTVHEKLKWAFLNYDINKDGYITKEEMLAIMKSIYDMMGRHTYPILREDAPAEHVERFFEKMD
RNQDGVVTIEEFLEACQ
KDENIMSSMQLFENVI

Fig.16 Continued

RAT P19 DNA (FIRST PASS, PARTIAL; CD:1-330)

TTTGAGGACTTTGTGGTTGGGCTCTCCATCCTGCTTCGAGGGACCGTCCATGAGAAGCTCAAGTGGGCCTTCAATCTCTA
CGACATCAACAAGGACGGTTACATCACCAAAGAGGAGATGCTGGCCATCATGAAGTCCATCTACGACATGATGGGCCGCC
ACACCTACCCTATCCTGCGGGAGGACGCACCTCTGGAGCATGTGGAGAGGTTCTTCCAGAAAATGGACAGGAACCAGGAT
GGAGTAGTGACTATTGATGAATTTCTGGAGACTTGTGAGAAGGACGAGAACATCATGAGCTCCATGCAGCTGTTTGAGAA
CGTCATCTAGGACATGTAGGAGGGGACCCTGGGTGGCCATGGGTTCTCAACCCAGAGAAGCCTCAATCCTGACAGGAGAA
GCCTCTATGAGAAACATTTTTCTAATATATTTGCAAAAAGTG

RAT P19 PROTEIN (PARTIAL)

FEDFVVGLSILLRGTVHEKWKWAFNLYDINKDGYITKEEMLAIMKSIYDMMGRHTYPILREDAPLEHVERFFQKMDRNQD
GVVTIDEFLETCQKDENIMSSMQLFENVI

Fig.17

MOUSE P19 DNA (CD: 49-819)

CGGGCTGCAAAGCGGGAAGSTTAGTGACGGTCCCTTTTCAGCAGCAGAGATGCAGAGGACCAAGGAAGCCGTGAAGGCATC
 AGATGGCAACCTCCTGGGAGATCCTGGGCGCATACCACTGAGCAAGAGGGAAAGCATCAAGTGGCAAAGGCCACGGTTCA
 CCCGCCAGGCCCTGATGCGTTGCTGCTTAATCAAGTGGATCCTGTCCAGTGTGCCCCACAAGGCTCAGACAGCAGTGAC
 AGTGAACCTGGAGTTATCCACGGTGCGCCATCAGCCAGAGGGCTTGGACCAGCTACAAGCTCAGACCAAGTTCACCAAGAA
 GGAGCTGCAGTCCCTTTACCGAGGCTTCAAGAATGAGTGTCCCACAGGCTGGTGGATGAAGACACCTTCAAACCTCATTT
 ATTCCCAGTTCTTCCCTCAGGGAGATGCCACCACCTATGCACACTTCTCTTCAATGCCTTTGATGCTGATGGGAACGGG
 GCCATCCACTTTGAGGACTTTGTGGTTGGGCTCTCCATCCTGCTTCGAGGGACGGTCCATGAGAAGCTCAAGTGGGCCTT
 CAATCTCTATGACATTAACAAGGATGGTTGCATCACCAGGAGGAGATGTGGCCATCATGAAGTCCATCTACGACATGA
 TGGGCCGCCACACCTACCCCATCCTGCGGGAGGATGCACCCCTGGAGCATGTGGAGAGGTTCTTTTCAGAAAATGGACAGG
 AACCAGGATGGAGTGGTGACCATTGATGTATTTCTGGAGACTTGTGAGAAGGATGAGAACATCATGAACTCCATGCAGCT
 GTTTGAGAACGTCATCTAGGACATGTGGGAGGGGACCCCACTGGTCAATTGCTTCTCAACCCAGAGSAGCCTCAATCCTGA
 CAGGAGAAGCCTCTATGAGAAACATTTTTCTAATATATTTGCAAAAAGTGAGCAGTTTACTTCCAAGACACAGCCACCGT
 CACACACAGACACAGACATACAGACACACACACACACACACATGGTTCCTCTGGCTTGGCCAAGGAAGTGGCAGCC
 AGAAGGCACCCCGCCTATTCTAGGTCAATAAAAAAGGCTGCCTCTGGGATGGCCAGCCCTGGCTAGATGTTACCCACA
 AGGAACTCAGAGATCGAGAGGACCAGGTCTACAAAGCTAAGGTCCCTGTGTCTTTTCTACCACTCGGGAGATCAAACCTAC
 TCCCTGCCTATGGACCCATGCTCTTAGGAAGCTCCCGAAAACCTCAAGGGGACAAAGAGGGGAGAGGTCTATAGGAAGAA
 ATGGTTTTGGAAGCTGGGCTTGCAGCCTTATGCTAATGATCACCTGGGGTCCCTGGAACCCGAGTGCCAGGCTACCTACTA
 TGCCGTGAGCTTAGATAGTGAGGGGCCATTGGACTAAGACCTCCTGTAAGAGTGGGGCAGGATTGAGGTTTTTGGAGAAA
 CTGAGGAAACAATTTGTCCATACCACTGGGTGAAGACTGCTGGCCAGTGGGAATGTGGCTGGTGGAGATTTCCCAACTTC
 CAGCACCAGGATGGCCTCTCAAGGTCTCTTTGATTCCCTGGGGAGATCACCTGGCTCATAGACTGACAACCAGGGAAC
 TGGGCTGAAATGGGAGGTCTGGTAGGGGGCATCCCCCTCCTTTTCCCTGGCCACTTGCCACCCAGTTCCCTTAACACAGTG
 GATCGGCCACACCTCTGTGGCTGCCCTTGAACAGACTCATCCCGACCAAGACAAAAAGCACTAACTCCTAGCAGCTCAG
 GCCAAGCCCACAAGGAAGGCTGGGTCCCTGCAGCCCTGATTCAGTGGCCGAGGAAGACGCTCAGACATCCATCCTGTA
 CCTCGGAGCCTTGGGGGTCTCACAGCCCTTTCCAGCCAGCTCGCCAACATTCCTAAAGCACAAACCTGCGGATTCTGCT
 TGCTTGGGCTGCGCCCTGGGGATTGAAGGCCACTGTTAACCCCTAAGCTGGAGCTAGCCCTGAGGGCTGGGGACCTGTGAC
 CAGGCAACAGGTGAGCAGACCCCTCAGGAGGAGAGAGAGCTGTTCCTGCCTCCCCAGGCCTCGCCAGAAGGAACAGTGTC
 CCAAGAAGCATGTTTTCTGGAGGAACATCCCCACAAAAGTACATTCCATCATCTGAAGCCCGGTCTCTGCTCAGGCCTGC
 CTCTGAAAGTCCACGTGTGTTCCCCAGAAGGCCAGCCCCAAGATAAGGGAGGTCTTAGAGGAAGGACAGGGTGACAACA
 CCCCTATACACAGGTGGACCCCCCTCTGAGGACTGTACTGACCCCATCTCCATCCTGACCGGGGCCTTCCTTTACCCGA
 TCTACAGACCACAGTTCTCCCTGGCTCAGGGACCCCTGTCCCCAGTCTGACTCTTCCCATCGAGGTCCCTGTCTTGT
 GAAAAGCCAAGGCCACGGGAAAAGGCCACCACTCTAACCTGCTGCATCCCTTAGCCTCTGGCTGCACGCCCAACCTGGAG
 GGGTCTGTCCCCCTTTCAGGGACACAGACTGGCCGCATGTCCGCATGGCAGAAGCGTCTCCCTTGGGTGCAGCCTGGAAG
 GGTGGTTTCTGTCTCAGCGCCACCAATATTCAGTCTATATATTTTAATAAAAGAACTTGACAAAGGAAAAAAAAAA
 AAAA

Fig. 18

>AI 352454 (partial) cds = 1-339

CACGAGGTGGAAAGCATTTCGGCTCAGCTGGAGGAGGCCAGCTCTACAGGCGGTTTCCTGT
 ACGCTCAGAACAGCACCAA
 GCGCAGCATTAAAGAGCGGCTCATGAAGCTCTTGCCCTGCTCAGCTGCCAAAACGTCGTCTC
 CTGCTATTCAAAACAGCG
 TGGAAGATGAACTGGAGATGGCCACCGTCAGGCATCGGCCCAGGCCCTTGAGCTTCTGGA
 AGCCCAGAGCAAATTTACC
 AAGAAAGAGCTTCAGATCCTTTACAGAGGATTTAAGAACGTAAGAACTTTCTTTTGGACTTT
 ACCTTCACACAATTCCCA
 GAGGAGCATTGAGAAATGAgaggaaaaggggaaaatatccattctatgagaagcccatcatatgtatatttcatact
 gatccttcccagataggaatataatcagtatctgtggactttgaatctctgtggcacacccatgctggcatactgtaatt
 gccattaaacaaanagtttttgagaaaaaaaaaaaaaaaaaaaaaaaaaaaaa

>AI352454

HEVESISAQLEEASSTGGFLYAQNSTKRSLKERLMKLLPCSAKTSSPAIQNSVEDELEMATVRHR
 PEALELLEAQSKFT
 KKELQILYRGFKNVRTFFLTLP SHNSQRSIEK

Fig. 19

P193 (AA349365) DNA (CD:2-127,partial)

TGAAAGGTTCTTCGAGAAAATGGACCGGAACCAGGATGGGGTAGTGACCATTGAAGAGTTCCTGGAGG
CTGTCAGAAGGATGAGAACATCATGAGCTCCATGCAGCTGTTTGAGAATGTCATCTAGGACACGTCCAAA
GGAGTGTCATGGCCACAGCCACCTCCACCCCCAAGAAACCTCCATCCTGCCAGGAGCAGCCTCCAAGAAA
CTTTTAAAAAATAGATTTGCAAAAAGTGAACAGATTGCTACACACACACACACACACACACACACAC
ACACACACACAGCCATTTCATCTGGGCTGGCAGAGGGGACAGAGTTCAGGGAGGGGGCTGAGTCTGGCTAG
GGGCCGAGTCCAGGAGCCCCAGCCAGCCCTTCCAGGCCAGCGAGGCGAGGCTGCCTCTGGGTGAGTGG
CTGACAGAGCAGGTCTGCAGGCCACCAGCTGCTGGATGTACCAAGAAGGGGCTCGAGTGCCCCCTGCAG
GGGAGGGTCCAATCTCCGGTGTGAGCCACCTCGTCCCGTTCTCCATTCTGCTTTCTTGCCACACAGTGGG
CCGGCCCCAGGCTCCCTTGGTCTCCTCCCCGTAGCCACTCTCTGCCCACTACCTATGCTTCTAGAAAGCCC
CTCACCTCAGGACCCAGAGGGACCAGCTGGGGGGCAGGGGGGAGAGGGGGTAATGGAGGCCAAGCCT
GCAGCTTTCTGGAAATCTTCCCTGGGGGTCCCAGGATCCCCTGCTACTCCACTNACCTGGAAGAGCTGG
GTACCAGGCCACCCACTGTGGGGCAAGCCTGAGTGGTGAGGGGCCACTGGGCCCCATTCTCCCTCCATGG
CAGGAAGGCGGGGGATTTCAAGTTTAGGGATTGGGTCTGGTGGAGAATCTGAGGGCACTCTCTGCCAG
CTCCACAGGGTGGGATGAGCCTCTCCTTGCCCCAGTCTTGGTTCAGTGGGAATGCAGTGGGTGGGGCIGT
ACACACCCCTCCAGCACAGACTGTTCCCTCCAAGGTCTCTTAGGTCCCGGGAGGAACGTGGTTCAGAGAC
TGGCAGCCAGGGAGCCCCGGGGCAGAGCTCAGAGGAGTCTGGGAAGGGGCGTGTCCCTCCTCTTCTGT
GTGCCCCCTCCATGGCCCAGCAGCTTGGCTGAGCCCCCTCTCCTGAAGCAGTGTGCGCCGTCCCTCTGCCTT
GCACAAAAGCACAAAGCATTCTTAGCAGCTCAGGCGCAGCCCTAGTGGGAGCCCAGCACACTGCTTCT
CGGAGGCCAGGCCCTCCTGCTGGCTGAGGCTTGGGCCAGTAGCCCCAATATGGTGGCCCTGGGGAAGA
GGCCTTGGGGGTCTGCTCTGTGCCTGGGATCAGTGGGGCCCCAAAGCCCAGCCCGGTGACCAACATTCA
AAAGCACAAACCCTGGGGACTCTGCTTGGCTGTCCCTCCATCTGGGGATGGAGAATGCCAGCCCCAAG
CTGGAGCCAATGGTGAGGGCTGAGAGGGCTGTGGCTGGGTGGTCAGCAGAAACCCCCAGGAGGAGAGA
GATGCTGCTCCCGCCTGATTGGGGCCTCACCCAGAAGGAACCCGGTCCCAGGCCGATGGCCCCCTCCAGG
AACATTCCCACATAATACATTCCATCACAGCCAGCCAGCTCCACTCAGGGCTGGCCCGGGGAGTCCCCG
TGTGCCCCAAGAGGCTAGCCCCAGGGTGAGCAGGGCCCTCAGAGGAAAGGCAGTATGGCGGAGGCCATG
GGGGCCCCCTCGGCATTACACACAGCCTGGCCTCCCCCTGCGGAGCTGCATGGACGCTGGCTCCAGGCTC
CAGGCTGACTGGGGGCTCTGCCTCCAGGAGGGCATCAGCTTTCCCTGGCTCAGGGATCTTCTCCCTCCC
CTCACCCGCTGCCCAGCCCTCCCAGCTGGTGTCACTCTGCCTCTAAGGCCAAGGCCTCAGGAGAGCATCA
CCACCACACCCCTGCCGGCCTTGGCCTTGGGGCCAGACTGGCTGCACAGCCCAACCAGGAGGGGTCTGC
CTCCCACGCTGGGACACAGACCGGCCGATGTCTGCATGGCAGAAGCGTCTCCCTTGGCCACGGCCTGGG
AGGGTGGTTCTGTTCTCAGCATCCACTAATATTTCAGTCCTGTATATTTAATAAAATAAACTTGACAAAG
GAAAAAAAAAAAAAAAAA

P193 PROTEIN (PARTIAL)

ERFFEKMDRNQDGVVTIEEFLEACQKDENIMSSMLFENV

Fig. 20

Human 9q genomic DNA sequences:

A. exon1 sequence (with introns included):

CGGGAGGAGAGAGGCAGCTCGGCTCGGCTCCGCGCTCAGCTCCGCTCTGCCTCCGGCTCTGCGCTCACCTGCTGCCT
AGTGTTCCTCTCCTGCTCCAGGACCTCCGGGTAGACCTCAGACCCCGGGCCCATTTCCAGACTCAGCCTCAGCCCCG
GACTTCCCCAGCCCCGACAGCACAGTAGGCCGCCAGGGGGCGCCGTGTGAGCGCCCTATCCCGGCCACCCGGCGCCC
CCTCCCACGGCCCCGGGCGGAGCGGGGGCGCCGGGGGCCATGCGGGGCCAGGGCCGCAAGGAGAGTTTGTCCGATTCC
CGAGACCTGGACGGCTCCTACGACCAGCTCACGGGTGAGTCAGTGACGTGGGGTTCGCGGGAGGGAGGGTGGATTCC
ATTCTCCAGACCTTCCGCTCTCCGACCCCGGCTGGCCCCGACCAACACTCTGCCCCATTCCAGGCACTCTTA
TGGCCGGTCTGGGCGGCAGGACACTGGGGTTCAAAGCCTTGGGTCCCGCAGGGGTGGGGAGGAACAGAAGAGGCA
GGTGTGGAGAGGCAGCAGGTGTGGGCGTATGTGACACAGGCTGAGAGGGTGTCTGGAGTGGGAGGTGTTACCGTGC
GTGAGCACCTGTCTATTCTGTGTGTGTGTGTGTGTGCGCGCACCTCCACAGCTGGTTGCCATGTGCCCTGGGC
TTGGTGACAGCTAGGGTGAGTGTGATTGTATGTGGCAGTGCAATTGTATGGTCTCGTCAGATGTTTGTGTTTGGCGTA
GGACCTGGTGTACTGATGAAGTTGTTTTGACCATGTGTCTYTATGTGCAACGATGTGTTGTGAGTGTGTAATCT
GTATGAAAGTGGTGTGTAACCTACCAGAATGTGTACGGCTCTACTTTAGGGTGGCTTGTCTCTTTG

B. Exon 2-11 sequence (with introns included):

AGCCNANTGGGTCNCCATGTGTATGCATCCTGTTTACTTAGGTCACATTTGTATATGTTGTGTAAGGAGTACCAGGT
CAATGTGTGTGTGTGTGTGAGCATGNATAAACGCCANCAGGTGTGAGTTANTGAATATCAAGCTGTCACTGGCACCC
ATCACTGTGATGTATTGTTTCATACATGTACNAACACGGCTGTCACTGTAGGTGTGTGTATRAGAGAGGTGTTCTT
ACCCAGGCAATCCTTGGGTTGGACATCATCNTGAGAGGTCCAGCCATGGCACTTGAGCCAAGGGTACTAGGTACGCA
AAGACATTGAGGCCACTGCCACCTCATCCTTGCCGCTCGCTGTACCCGGCCACGTCCCATTAACCAAGTGCNTGA
GCCTCACCTCTATGGAATCCTGGGCTCCCTTAACCCGATTTCAACACCCCTTGCCATTCCTTTCTCCCTTAATT
CCTCCCCAGCCCGGTCCCAGATGGGGTTGATTGTGACTGGCGGGAGGGGACAGGGAACAGAGGACCCCGGGA
GTTAATGTGCTTCTGGGTTCTCTCTTNCAGGCCACCTCCAGGGCCCACTAAAAAGCGCTGAAGCAGCGA
TTCTCAAGCTGCTGCCGAGCTGCGGGCCCCAAGCCCTGCCCTCAGTCAGTGAAAGCAAGTGCCCTCTCATGTGCTTC
CCGGGGCGGGCTCGATGTGTGCGTGCCTGTCTGTGCATGANTGTGTGCGCGTGTGCCCCAGGCTGCRAGTGTGKCS
CATGYTCCAGGCTTGCATGTGTGGGGGGCGTGCCCCAAGCCTKSGTGTGTTGGGGGTGGGGCTGCCCAVGCCTGT
GCGTGTGTATGTGTGTGCATGTGCGCRGAGCGTRCCCCAGACCGCGTGTGTGTGTGTGGGGCGTGCCCTACCCC
TGCATGTGTGTGAGGGCGTGCCCCAKGCCCKGGCGNGTTGTTTGTGTGTATGGGAAGGCGTACCGCACGCCTGC
GTGTGGGGGAGGGCGTGCCCCAGAGCCTGCGTGCGTGTGTGTGTGTGTGTGTGTGTGTGGGCGTGACCAGCG
TGGCGAGGGCGGGTGCTGGCAAGGCTGGAGCATAAGNNGGCGNGGCTACATGTGTGNGTGTACGNCTGAAGCCAGCG
TGTGTGGGCGTGGTCAGTTGGNAGCGGGTGTGTGTACCGCTCCCGCAAACTGTGGGACCCGAGAGTGTGGGTGTG
ACCATTGTGACCAGNTGAGGCCTGAGCCTGTGTAGCTGTGGCGGCTGTGTAGACCAGGCGGCGTGAGGGTCTGT
ATGTGGCTTAGCTGGGTAGTGTCTTCAACTCCGTGCGGCGGCCCCCTTCCCCACCGTGTTTTGGACCCCTGATGTG
TGTTGCCATATGCCCCGACAGGATGGTGACAGGTGTAGAGGATGGCGGCTGCCCTCCTCCAGCAGGGGATTTTGG
GTTTTCTGTGCCAGCCTGCTCCCTGCTGAAGTGTATCCAGTTGAGTGACCTCGCTTTGTCTCTAGGTCTCCATTT
CCTCAGTTGGGCTTGCCCCACCTCATAGGATCATACTGCATTTTGCAAACCATAAAGGCCCGCTTTGTAGTTATTTG
AGCATGCTGTTGTGTGGACTTAGATGGGTCCACACGGGGGTGGATTCCGARAAGGACAGGCGTGAGTCCCGCAAG
CTTGTGTGCATGGGGTCCGTTTCGTGTGTGTCTGTGCTGGTTGGGTGTGCCTTTGCACGGGCTGGGTGTGAGGTTT
GCTCTGAGTGTGAGGGGCCAGGTGTGTGTATGCAGTTGGCCGGGTCTTCCGCTTTCTCGGTGWCAGTTCCGCTCCCTT
CAGCATTAGCCGCCCCAGCCTCCCTCCGCCCCACAGACCCCGCTGCTGGACCCAGGTGACTTACGCTCCTGGTGG
GGGCGGGCGGGCAGGGCGGCTTTGCCATCTTGGGGTGGGGGGCACTTGCCCTGGGGGCTGGACGTTGGGGCGGGG
CAGGATTGAGATGGGGCCGGGGGTGGGGTCTGGATGGAGGTTGGCTGAGCTGGGCGGGGCATGGCTCAGGCATGGCT
GGGATAGATGGGGTGGGCGGGCGAGGGGAGGGGCTGGGTGGGACGAGGGGAGGGTTTGGGCGGGGCAAGGCTGGG
GCTGGGCGGATCTGAGTTGGTCCCCAAGGCCCGAGCTCTGACCCCTCAGACGCCCCCTCTTGAACCTGGCTTTTCCC
ACTCCTCCCTTTCTAAAACGAAGATGCGGGCTGGGGGCTTCCCCCTCAACGAGGGATCGAGGGCCGCGGGGCGAGCA
CTGAGTCGGATCCCTGGCTCTGGGGCCAGGCCAGGCTTGGCCCGTGATAGACCTCGAAGATGGCCATCATCTTTT
CTCCTTACCTCAGTGTCTTGGCTCGGGGCCAGGGAACCTGGCAGCCTGGTCTCCGGCATCGGATGGGACCGGGGG
CGGGGAGGGGGTGAATGGGGCAGTGATTTGAAGAGGGGTGCGGGAGGCTGGGCATGAGGCGCGGCTGTCTCACCGC
TCCCGCAGACAGCGTGGACGATGAATTTGAATGTCCACCGTGTGTACCGGCTGAGGGTCTGGAGCAGCTGCAGG
AGCAAACCAATTACGCGCAAGGAGTTGCAGGTCTGTACCGGGCTTCAAGAAGCTGAGTGCGNGGGCGAGGCCAA

Fig. 22

ACTCAGCGNGGGTGGGACAGGAGGACCCAANCCGGTCCANATTTTTCCCANAAAGCATGGCTTNGATGCTTGAGGNG
 CGGGCGGAAGGGAGGCAAGGCCCTGAGACTGAACTTCTAGCTGGAGGTTCTGGGGCGGGGCCAGAACGRAAGTGGCG
 CCTGTAGACTGTGAGTTTCGTTCCATGTTTTTTATTTGTGCACTGGGAAAGAAGTCTTCCCTCCCATCACATGAGCC
 ACGTGGTGAGTCCCTCTGGAGGCTTGAAGATTATCCCCCTCCCTGGGAGTCTTGGGCCATGGAGGGTGGGGGCGGTGA
 ACGGAAGGGGATTTTGTCTCTGCCCTCAGCCTGGTGCCCTCTCCTTCCAGGAATGTCCAGCGGAATTGTCAATGAG
 GAGAACTTCAAGCAGATTTACTCCCAGTCTTTCTCAAGGAGGTGAGGGGACAAGGCCCAAGGGGAAGCAGTTGTC
 CTTCTCTAGGCTGAGGGAGGGAGGGATTCTGGAGGAGCTGGGAATGCCAAGGTGATGGGGGTATGGGGAGCTCCTT
 AGAGGGAGGAAGTCCCTCTCTGTGTGGAAGCCAACCTTCTCCACACTCACCTGCAGACTCCAGCACCTATGCCACTT
 TTCTCTTCAATGCCTTTGACACCAACCATGATGGCTCGGTGAGTTTGTAGGTGAGCTGGGCGAGGTGGGCCAGGGAA
 GCCTGTTTCTCGAGTTTCAAGGGCCAGGATCTCCAGGCCAAACCCAGAGAAGGAGTTGGGTGAAGAGKACCCGAGGAC
 ACAGCTCCCTTCTGCTTCTTCCCAGGACTTTGTGGCTGGTTTGYCCGTGATTCTTCGGGGAACTGTAGATGACAGG
 CTTAATTGGGCTTCAACCTGTATGACCTTAAACAAGGACGGCTGCATCACCAAGGAGGTGCAGGGCAACTGAAGGGC
 TGGGGGTCTGTGGCGGTGATGGGGTGGCGTGCAKAGGGTGTGGGAGGGAAATATGACCCACATATGCCACACAAGC
 AATGGGATCAAGGAGGCTGGAGGCTCTGAGGAAGGATCCTCTTCTCTTGGCCTAACAGGAAATGCTTGACATCA
 TGAAGTCCATCTATGACATGATGGGCAAGTACACGTACCCCTGCATCCGGGAGGAGGCCCAAGGGAACACGTGGAG
 AGCTTCTTCCAGTACTTGGGAGTGGGTATGGCTGGAGGGCCCTGGAGTGAAGGGAAGAAGGCCAAGAACCAGCAGG
 GAACTCACCTGACTTCTGTCTGCTCTCTCTTGGCCATCCCTCCTGTTCTCCTTGCCTGACCACCTTCTTGCAGAAGA
 TGGACAGAAACAAGGATGGGTGTGGTGACCAATTGAGGAATTCATTGAGTCTTGTCAAAAGGTACAGCTCCCTGCCCTC
 TACATTACCCTGACCTGGACTCAGGCCTGATTTAGTAATGCAGGGAAGGCTTCTTTGGGAAGAATACCACCTTCCC
 ACCTCACCCCATATTTCAATCCTATTCTTGTGGGAGGCTTACCCCTTCCCTACCTCAGGTCTCTCTGGGCATCT
 CTTTCTCTGTGCTTTTGAATGTCCCCGTCTGTGACTCAAGTGTCCCTCTCACTGTCTCTGATAAAGCTCCTTCTCT
 TTCTCTCTTCAATCTGCCTCGCTCACATCATGGCCACAGGATGAGAACATCATGAGGTCCATGCAGCTCTTTGAC
 AATGTCTATAGCCCCCAGGAGAGGGGGTCACTGTTTCTTGGGGGGACCATGCTCTAACCCCTAGTCCAGGCGGACCT
 CACCCCTTCTTCTTCCCAGGTCTATCCTCATCTACCCCTCCCTGGGGGGTGGAGGGATCCAAGAGCTTGGGGATTGAG
 TAGTCCAGATCTCTGGAGCTGAAGGGGCCAGAGAGTGGGCAGAGTGCATCTCGGGGGGTGTTCCCAACTCCCACCAG
 CTCTCACCCCTTCTTGCCTGACACCCAGTGTGAGAGTGGCCCTCCTGTAGGAATTGAGCGGTTCCCCACCTCCTA
 CCCCTACTCTAGAAACACACTAGACAGATGTCTCTGCTATGGTGTCTCCCCCATCCCTGACCTCATAAACATTTCC
 CCTAAGACTCCCTCTCAGAGAGAATGCTCCATTTCTTGGCACTGGCTGGCTTCTCAGACCAGCCATTGAGAGCCCTG
 TGGGAGGGGGACAAGAATGTATAGGGAGAAATCTTGGGCTGAGTCAATGGATAGGTCTTAGRAGGTGGCTGGGGT
 GAGAATAGAAGGGCTGGACAGATTATGATTGCTCAGGCATACCAGGTTATAGTCCAAGTTCACAGGTCTGCTAC
 CACAGGCCATCAAAATATAAGTTTCCAGGCTTTGAGAGAGACCTTGTCTCCTTAGAAATGCCCCAGAAATTTCCAC
 ACCCTCCTCGGTATCCATGGAGAGCCTGGGGCCAGATATCTGGCTCATCTCTGGCATGCTTCTCTCTCTCTTTCC
 TGCATGTGTTGGTGGTGGTGTGGTGGGGGAATGTGGATGGGGGATGCTCTGGCTGATGCTGCCAAAATTTTCATCC
 CACCCCTCTTGGCTTATCGTCCCTGTTTTGAGGGCTATGACTTGAGTTTTTGTTCCTCATGTTCTCTATAGACTTGGG
 ACCTTCTGAACTTGGGGCTATCACTCCCCACAGTGGATGCCTTAGAAGGGAGAGGGAAGGAGGGAGGCAGGCATA
 GCATCTGAACCCAGTGTGGGGCATTCACATAGAATCTTCAATCAACCTGGGCTCTCCCCACCCACCCAGATAACC
 TCCTCAGKTCCTAGGCTCTTCTCTYGCTTGACTCAATCTACCCAGAGATGCCCCCTTAGCACACCTAGAGGGCAGGG
 ACCATAGGACCCAGGTTCCAACCCATTGTGACACCCAGCCATGCGGCCACCCCTTAGCACACCTGCTCGTCCCA
 TTTAGCTTACCCTCCCAGTTGGCCAGAATCTGAGGGGAGAGCCCCCAGAGAGCCCCCTTCCCCATCAGAAGACTGTT
 GACTGCTTTGCATTTTGGGCTCTTCTATATATTTTGTAAAGTAAGAAATATACCAGATC : TAATAAAACACAATGGC
 TATGCACAGGCTGCCGTCTCTGCCCTTTGTCCCTCCACCTACAAATACTACACAACCCCTAACGAATGCACCTGCA
 GCCTTTTAGATCCCCAAGAAAGTGCTTTCTTTTCCATAGTTGGCCATACCTTGGCATGAGACTGAGACACAGGCTC
 TGGAATGGTTGGAAACCCACCAACCTCAGGCCCCACATGAATCTCCCTCCCACACAGCCTGAGAGGAGACAAGGA
 AGGAAGGACAGGACACTGATGTCCCGAAGACTGTGCCAAGCAAGCTGTTTTTTAGCTGACATTCTTACAAGTTGAAT
 CACAGATTTCTAATTTACAGACTTTTTAGTTAATCTCAAAGTGCTTTCTTTTGGGGGCTCCTTTAAGTTCYTTCT
 TTTTTTTTTTTTTT

Fig. 22 Continued

>monkey KChIP4 cds = 265

gtcgacccacgcgtccggtgctgtggagcgggggggagccccgccagccaaatgccaggatcagcatgagaggctgg
acttttagtccaggtctgtcctcaccgggggacgcggcctttgcagggtgcagctgcgaggaactgctcacttttttc
cccttgcaagtctttgttccaagcctgacgttgctacgattctgttaactccctccactccaaaggggtctggaggc
tgggatgctctgccagctcagaggATGTTGACTCTGGAGTGGGAGTCCGAAGGACTGCAAACAGTGGGTA
TTGTTGTGAT
TATATGTGCATCTCTGAAGCTGCTTCATTTGCTGGGACTGATTGATTTTTTCGGAAGACAGCGT
GGAAGATGAACTGGAGA
TGGCCACTGTCTAGGCATCGGCCTGAGGCCCTTGAGCTTCTGGAAGCCCAGAGCAAATTTACC
AAGAAAGAGCTTCAGATC
CTTIACAGAGGATTTAAGAACGAATGCCCCAGTGGTGTGTTAATGAAGAAACCTTCAAAGA
GATTTACTCGCAGTTCTT
TCCACAGGGAGACTCTACAACATATGCACATTTTCTGTTCAATGCGTTTGATACGGACCACA
ATGGAGCTGTGAGTTTCG
AGGATTTTCATCAAGGTCTTTCCATTTTGCTCCGGGGGACAGTACAAGAAAACTCAATTGG
GCATTTAATCTGTATGAT
ATAAATAAAGATGGCTACATCACTAAAGAGGAAATGCTTGATATAATGAAAGCAATATACG
ACATGATGGGTAAATGTAC
ATATCTGTCTCTCAAAGAAGATGCACCCAGACAACACGTCGAAACATTTTTTCAGAAAATGG
ACAAAAATAAAGATGGGG
TTGTTACCATAGATGAGTTCATTGAAAGCTGCCAAAAAGATGAAAACATAATGCGCTCCATG
CAGCTCTTTGAAAATGTG
ATTTAActtgtaactagatcctgaatccaacagacaaatgtgaactattctaccacccttaaagtcggagctaccactt
ttagcatagattgctcagcttgacactgaagcatattatgcaaacaagctttgttttaataaaagcaatcccaaaaga
tttgagtttctcagttataaatttgcatcctttccataatgccactgagttcatgggatgttctaactcatttcatactc
tgtgaatattcaaaagtaataagaatctggcatatagttttattgattccttagccatgggattattgaggctttcacata
tcagtgatttttaaataaccagtgtttttgctctcatttgatgtattcagtcctaggattttgaatggtttttctaata
actgacatctgcatttaatttccagaaattaataattttcatgtctgaatgctgtaattccatttatatactttaagt
aaacaaataagattactacaattaacacatagttccagtttctatggccttcccttcccaccttctattataaattaat
tttatctgggtatttttaaacattttaaatttatcatcagatatcagcatatgcctaattatgcctaataaagacttaata
agcatttaattttccatcatacattatagccaaggcctatatactatataaattttggatttgtttaattcttacagggt
gttttccattgtatcatcaagtggaggttcaagcggcatcaacaaaacaaggatgtttacagacatatgcaaaggggtc
aggatatctatcctccagtatatgttaattgcttaataacaagtaatacctaacagcattaaaggccaaatctgtcctctt
ccctgacttcccttacagcatgtttatattacaagccattcaggggacaaagaaaccttgactacccactgtctactagg
aacaaacaaacagcaagcaaaattcactttgaaagcaccagtgggtccattacattgacaactactaccaagattcagta
gaaaataagtgctcaacaactaatccagattacaatatgatttagtgcataaaaattccaacaattcagattattttt
aatcatctcagccacaactgtaaagttgccacattactaaagacacacacatcgccctgttttgtagaaatatcacaaa
gaccaagaggctacagaaggaggaaatttgcaactgtctttgcaacaataaatcaggtatctattctggtgtagagatag
gatgttgaaagctgccctgctatcaccagtgtagaattaagagtagtacaatacatgtacactgaaatttgccatcgcg
tgtttggtgaaactcaatgtgcacattttgtatttcaaaaagaaaaataaaagcaaaataaaatggtwawaamwmwaaa
aaaaaaaaaaaaa

>monkey KChIP4

MLTLEWESEGLQTVGIVVIICASLKLHLGLIDFSEDSVEDELEMATVRRHRPEALELLEAQSKFT
KKELQILYRGFKNE
CPSGVNEETFKEIYSQFFPQGDSTTYAHFLFNAFDTDHNGAVSFEDFIKGLSILLRGTVQEKLNW
AFNLYDINKDGYIT
KEEMLDIMKAIYDMMGKCTYPVLKEDAPRQHVETFFQKMDKNKDGVTIDEFIESCQKDENIM
RSMQLFENVI

Fig. 23

>monkey KChIP4 C terminal splice variant cds = 265-966

gtcgacccacgcgtccggtgcgctgtggttgcggggggagccccgccagccaaatgccaggatcagcatgagaggctgg
 acttttagtccaggtctgtcctcaccgggggaccgcccggccttgcagggtgcagctgcgaggaactgctcacttttttc
 cccttgcaagtctttgtccaagcctgacgttgctacgattctgtaattaactccctccactccaaaggggtctggaggc
 tgggatgctctgccagctcagaggATGTTGACTCTGGAGTGGGAGTCCGAAGGACTGCAAACAGTGGGTA
 TTGTTGTGAT
 TATATGTGCATCTCTGAAGCTGCTTCATTTGCTGGGACTGATTGATTTTTTCGGAAGACAGCGT
 GGAAGATGAACTGGAGA
 TGGCCACTGTTCAGGCATCGGCCTGAGGCCCTTGAGCTTCTGGAAGCCCAGAGCAAATTTACC
 AAGAAAGAGCTTCAGATC
 CTTTACAGAGGATTTAAGAACGAATGCCCCAGTGGTGTGTTAATGAAGAAACCTTCAAAGA
 GATTTACTCGCAGTTCTT
 TCCACAGGGAGACTCTACAACATATGCACATTTTCTGTTCAATGCGTTTGATACGGACCACA
 ATGGAGCTGTGAGTTTCG
 AGGATTTTCATCAAAGGCTTTTCCATTTTGCTCCGGGGGACAGTACAAGAAAACTCAATTGG
 GCATTTAATCTGTATGAT
 ATAAATAAAGATGGCTACATCACTAAAGAGGAAATGCTTGATATAATGAAAGCAATATACG
 ACATGATGGGTAAATGTAC
 ATATCCTGTCTCAAAGAAGATGCACCCAGACAACACGTCGAAACATTTTTTCAGGCTGTTT
 TCCATTGTATCATCAAGT
 GGAAGTTCAAGACGGCATCAAACAAAACAAGGATGTTTACAGACATATGCAAAGGGTCAGG
 ATATCTATCTCCAGTATA
 TGTTAAtgcttaataacaagtaatcctaacagcattaaaggccaaatctgtcctctttccctgacttccttacagcatg
 tttatattacaagccattcagggacaaagaaaccttgactacccactgtctactaggaacaaacaaacagcaagcaaaa
 ttcactttgaaagcaccagtggttccattacattgacaactactaccaagattcagtagaaaataagtgtcaacaacta
 atccagattacaatatgatttagtgcatacaaaattccaacaattcagattatttttaatcatctcagccacaactgta
 aagttgccacattactaaagacacacacatcgctccctggtttgtagaaatatcacaagaccaagaggctacagaaggag
 gaaatttgcaactgtctttgcaacaataaatcaggtatctattctggtgtagagataggatggtgaaagctgccctgcta
 tcaccagtgtagaaattaagagtagtacaatacatgtacactgaaatttgccatcgcggtgtttgtgtaaaactcaatgtgc
 acattttgtattttcaaaaagaaaaataaaagcaaaaataaaatggttwawaamwmwaaaaaaaaaaaaaaaaaaaa

>monkey KChIP4 C terminal splice variant

MLTLEWESEGLQTVGIVVIICASLKLHLGLIDFSEDSVEDELEMATVRHRPEALELLEAQSKFT
 KKELQILYRGFKNE
 CPSGVVNEETFKEIYSQFFPQGDSTTYAHFLFNAFDTDHNGAVSFEDFIKGLSILLRGTVQEKLNW
 AFNLYDINKDGYIT
 KEEMLDIMKAIYDMMGKCTYPVLKEDAPRQHVETFFQAVFHCIKWKFKTASNKTRMFTDICK
 GSGYLSSSIC

Fig. 24

KChip1_1v -----MGAVMGTF-----SSLQTKQ----RKP-----
 KChip2_9q1 MRGQGRKESLSDSRDLGSDYDQITGHPPGPTKKALKQRFLLKLLPCCGPQALPSVSETLAA
 KChip3_p19 --MQPAKEVTKAS---DGSLLGLGH-----TPLSKKEGLKWQRPRLSRQALMRCCLVKWI
 KChip4_352 ---MLTLEWESEGLQTVGIVVITCAS----LKLLHLLGLIDFSE-----
 KChip4_231 ---MLTLEWESEGLQTVGIVVITCAS----LKLLHLLGLIDFSE-----
 hsnscspara ----HEVESISAQLEEASSTGGFLYAQN-STKRSIKERLMKLLPCS-----

KChip1_1v -----SKDKLEDELEMTMCHRPEGLEQLEAQTNFTKRELOVLYRGEKNECPS
 KChip2_9q1 PASLRPHRPRLLDPDSVDBEFELSTVCHRPEGLEQLEAQTNFTKRELOVLYRGEKNECPS
 KChip3_p19 LSSTAPQ-----GSDSSDSELELSTVRHOPEGLDOLQAQTKFTKKELQSLYRGEKNECPT
 KChip4_352 -----DSVEDELEMATVRHRPEALELLEAQSKFTKKELQILYRGEKNECPS
 KChip4_231 -----DSVEDELEMATVRHRPEALELLEAQSKFTKKELQILYRGEKNECPS
 hsnscspara -AAKTSSP---AIQNSVEDELEMATVRHRPEALELLEAQSKFTKKELQILYRGEKNECPS

KChip1_1v GVVNEDTFKQIYAQFFPHGDASTYAHYLFNAFDTTQTGSKVFEDFVTALSTLLRGTVHEK
 KChip2_9q1 GIVNEENFKQIYSQFFPOGDSSTYATFLFNAFDTNHDGVSFEDFVAGLSVLLRGTVDDR
 KChip3_p19 GLVDEBTFKLIYAQFFPOGDATTYAHFLFNAFDDGNGAHTFEDFVVGSLTLLRGTVHEK
 KChip4_352 GVVNEETFKETYSQFFPOGDSTTYAHFLFNAFDDHNGAVSFEDFVKGLSTLLRGTVQEK
 KChip4_231 GVVNEETFKETYSQFFPOGDSTTYAHFLFNAFDDHNGAVSFEDFVKGLSTLLRGTVQEK
 hsnscspara FETLPSHNSQRSIEK-----

KChip1_1v LRMTFNLYDINKDGYITKEEMMDIMKATYDMMGKYTYPVLKEDTPROHVDVFFQKMD
 KChip2_9q1 LNWAFNLYDINKDGYITKEEMMDIMKATYDMMGKYTYPALREEAPREHVESFFQKMD
 KChip3_p19 LKWAFFNLYDINKDGYITKEEMMDIMKATYDMMGRHTYPTLREDAPAEHVERFEKMD
 KChip4_352 LNWAFNLYDINKDGYITKEEMMDIMKATYDMMGKCTYPVLKEDAPROHVEFFQKMD
 KChip4_231 LNWAFNLYDINKDGYITKEEMMDIMKATYDMMGKCTYPVLKEDAPROHVEFFQKMD
 hsnscspara -----

KChip1_1v ---KNKDGIVTLDEEFLESCQEDDNIMRSMOLFENVM
 KChip2_9q1 ---RNKDGIVTLDEEFLESCQKDNIMRSMOLFENVM
 KChip3_p19 ---RNQDGIVTLDEEFLEACQKDNIMSSMOLFENVM
 KChip4_352 ---KNKDGIVTLDEEFLESCQKDNIMRSMOLFENVM
 KChip4_231 IKWKFKTASNKTRMTDICKGSGYLSSTIC-----
 hsnscspara -----

Fig. 25

Rat 33b07 protein

MNGVEGNNELPLANTSTLSALVPEDLDLKQDQPLSEETDTVREMEAAEAGAEGGASPDSEHCDPQLCLRVAENGCAAAAG
 EGLEDGLSSSKCGDAPLASVAANDSNKNGCQLAGPLSPAKPKTLEASGAVGLGSQMMPGPKTKVMTTKGAISATTGKEG
 EAGAAMQEKKGQVQKEKKAAGGGKDETRPRAPKINNCMDSLAIDQELSNVNAQADRAFLQLERKFGMRRLHMQRRSFII
 QNIPGFVWTAFRNHPQLSPMISGODEDDMMRYMINLEVEELKHPRAGCKFKFIFQSNPYFRNEGLVKEYERRSSGRVVSLS
 TPIRWHRGQEPQAHIHNRREGNTIPSFFNWFSHSLLEFDRIAELIKGELWSNPLQYYLMGDGPRRGVRVPPRQPVESPR
 SFRFQSG.

Rat 33b07 DNA (coding: 85-1308)

GGTGGAGCTAAGCACTCACTGCGGTGCTGCCCTGCGTCTGCAGAGAACAAAGGAAAGCTTCTCTGCAGGGCTGTCAGCTGC
 CAAAATGAACGGCGTGGAAGGGAACAACGAGCTCCCTCTCGCTAACACCTCGACCTCCGCCCTTGTCCCGGAAGATCTGG
 ATCTGAAGCAAGACCAGCCGCTCAGCGAGGAACTGACACGGTGGGGAGATGGAGGCTGCAGGTGAGGCCGCTGCGGAG
 GGAGGCGCGTCCCCGATTCCGAGCACTGCGACCCCACTCTGCCCTCCGAGTGGCTGAGAATGGCTGTGCTGCCGAGC
 GGGAGAGGGGCTGGAGGATGGTCTGTCTTCATCAAAGTGTGGGGACGCACCCCTTGGCGTCTGTGGCAGCCAACGACAGCA
 ATAAAAATGGCTGTGAGCTTGCAGGGCCGCTCAGCCCTGCTAAGCCAAAACTCTGGAAGCCAGTGGTGCAGTGGGCCTG
 GGGTCGCAGATGATGCCAGGGCCGPAAGAAGACCAAGTAATGACTACCAAGGGCGCCATCTCTGCGACTACAGGCAAGA
 AGGAGAAGCAGGGCGGCAATGCAGGAAAAGAAGGGGTGCAGAAAGAAAAAAGGCAGCTGGAGGAGGGAAGACGAGA
 CTCGTCTAGAGCCCTAAGATCAATACTGCATGGACTCCCTGGAAGCCATCGATCAAGAGCTGTCAAATGTAAATGCG
 CAAGCTGACAGGGCCTTCTCCAGCTGGAACGCAAAATTTGGGCGGATGAGAAGGCTCCACATGCAGCGCCGAAGTTTCAT
 CATCCAAAACATCCAGGTTTCTGGGTACAGCGTTTCGGAACACCCGCAACTGTCACCGATGATCAGTGGCCAAGATG
 AAGACATGATGAGGTACATGATCAATTTAGAGGTGGAGGAGCTTAAGCACCCAAGAGCAGGGTGCAAAATTTAAGTTCATC
 TTCCAAAGCAACCCCTACTTCCGAAATGAGGGGCTGGTCAAAGAGTACGAGCGCAGATCCTCAGGTGAGTGGTGTGCT
 CTCTACGCCAATCCGCTGGCACCAGGGGTCAAGAACCCAGGCCCATATCCACAGGAATAGAGAGGGGAACACGATTCCCA
 GTTCTTTCAATTGGTTCTCAGACCACAGCCTCCTAGAATTCGACAGAATAGCTGAAATTATCAAAGGGGAGCTTTGGTCC
 AATCCCCCTACAATACTACCTGATGGGCGATGGGCCACGCAGAGGAGTTCGAGTCCACCAAGGCAGCCAGTGGAGAGTCC
 CAGGTCCCTTCAGGTTCAGTCTGGCTAAGCTCTGCCCTCGTGAGAAGCTTTACAGAAGAGTCTTACCACCTTCTCAGC
 TTGGCTAGCAGCATGCAGCCTTCTGTCTGCTTTCTCTCTTGGATTGTGTCTTTGGTTCTTCTAAGTCTCCGGTAGTT
 TCAAGGTTGTGGCTTCCAAGTCTTTGCTCTTCTTTCTTCTTGGCCATCACGATGTCCTGCATAGTGTAAATGGTGTTCCAA
 GTGCTGTGATCTTCTAGGTTTTTTGTTTTCTTTTTTAAAGTGGTTCTCTATCAAAAGAAAGCTTGACATATCCTTACCAA
 GAACTAGCCAGATTTCTACTGTGTTCCTGATATCTATGTACTGTGAAGAACTGTGAGTTTCGCCACTGCAAGATGGGAC
 TGTATCCCAATCCAGCCATCAGCCCAACAGGACATTCCAAGCTGTACCAACTGATCCTAGCTGTCTTCTGGGCCCTTG
 CCAATTTACCCTGCTTTTTATCTATAGAATGAGCAGGTGGCTGGTAGGTGACTACTAGGTAAGAGTGAAGTATTAGGTGAG
 GAGTGTCTTCTGTACCAATGCTTCTGTACCAATGCATCATGATCAGCTTGGATCAGCTACTGACTGTCTGATATTTT
 TAACCCCAACACAAAAA

Fig. 26

TGGGTGGTGTCTAGACGTTTCGGGGCAGAGCTCGGCCGCTCGCGAGGACAAGGAACCTCCCTCTCCCACTAGTCTGACTTC
 TTCCAAAATGAGCGGCTGGATGGGGGCAACAAGCTCCCTCTCGCCCAAACCGCGGCGCTGGCTGCTCCCGACCATGCCT
 CAGGAGATCCGGACCTAGACCAAGTCCCAAGGGCTCCGTGAAGAAACCGAGGCGACACAGGTGATGGCGAAACACAGGTGGG
 GCGAGCCTGGAGACCGTTGCGGAGGGGGGTGCATCCAGGATCCTGTGCACTGTGGCCCCGCGCTCCGCGTCCCAGTTGC
 CGGGAGTCGCGGCGGTGCAGCGACCAAAGCCGGGCAGGAGGATGCTCCACCTTCTACGAAAGGTCTGGAAGCAGCCTCTG
 CCGCCGAGGCTGCTGACAGCAGCCAGAAAAATGGCTGTCACTTTGGAGAGCCCCGTGGCCCTGCTGGGCGAGAAGGCTCTA
 GAAGCCTGTGGCGCAGGGGGCTTGGGGTCTCAGATGATACCGGGGAAGAAGGCAAGGAAGTACAGCTAAAAAACGCGC
 CATCTCGGCAGCAGTGGGAAAGGAGGAGTACAGGCGCCGCGATGGAGGAAAAGAGGTAGTGCAGAAGGAAAAAAGG
 TCGCAGGAGGGGTGAAAGAGGAGACACGGCCAGGGCCCCGAAGATCAATAACTGCATGGACTCACTGGAGGCCATCGAT
 CAAGAGTTGTCAAACGTAAATGCCAGGCTGACAGGGCCTTCCTTCAGCTTGAGCGCAAGTTTGGCCGCATGCCAAGGCT
 CCACATGCAGCGCAGAAGTTTCATTATCCAGAATATCCAGGTTTCTGGGTTACTGCCTTTTCGAAACCACCCCCAGCTGT
 CACCTATGATCAGTGGCCAAGATGAAGACATGCTGAGGTACATGATCAATTTGGAGGTGGAGGAGCTTAAACACCCAG
 GCAGGCTGCAAATTCAGTTTCATCTTTTCAGGGCAACCCCTACTTCCGAAATGAGGGGCTTGTCAAGGAATATGAACGCAG
 ATCCTCTGGCGGGGTGTGTCTCTTTCCACTCCAATCCGCTGGCACCAGGCGCAAGACCCCGAGCTCATATCCACAGAA
 ACCGGGAAGGGAACATCTCCCTAGTTTCTTCAACTGGTTTTACAGCCACAGCCTTCTAGAATTCGACAGAATTGCAGAG
 ATTTACAAAGGAGAATCTGGGCCAATCCCTCAACAATACTACCTGATGGGTGAAGGGCCCCGTAGAGGAATTCGAGGGCC
 ACCAAGGCAGCCAGTGGAGAGCGCCAGATCCTTCAGGTTCCAGTCTGGCTAATCTCTGTCTGTGAGAAGCTTCTGCACA
 AGTTTCCTTACCACCTCCTCTTGGACCTATGCTTGGCCAACAGCATGCAGTCTTCCATCTGCTTTTCTCTTCACTGTGG
 ATTATCTTTTCTTTGGTTCTAAATCTTCAGTAATCGGTTGCAAGATTGTTGGCTTACCTGCCTGTGCCATTCTTCTCT
 GGGCCTTCATGCTTTTCTGCATTGTGTTAACATGTTTCAAGTGCAATGGCCTTCTACGGCTTCTATGCCAAGCGTATGATA
 CTATAGATATAGTGATACCTACTGCCTTCTTTTGCATGGCTTGGACCTATCTGTGACCATGCTCTTCCCAATTTAAG
 TGGTTCTGTACCAAAAGAACTTGTATACATTTTACAAAATAACTTATGGGCTTCTACTTTATGCTGGCTGTGTCTGT
 ATACCCATGTACTTATGTGAAGTATTTGGGTATTACCACTGCAAGACAAAACCTGATATCTTAAACCGGCCATCAACCCA
 AATTGGACATTCCAGACTACCACCAACTGGATCCAGCTGCCTTCTGGGCTTGTGCCATCCACCCTACTGGTTATCTGA
 TAGAACAGCTGGTGGCTGATGGGTGACTGCTAGGCGTGACTGAGGTAATAGATGAAAAGTGTCTATGTTATCACATTG
 GTTTTCTGTACCTTTTGGTTACTCTACGTCATGACCAGCTGCTGGTGAGTATGAAGCCTGTGCTATAGCCCACCCTACT
 CACTCTCACCTTCTGGTTGAACTTTGCTTAGGCCACCATTGTCTGCCTCATCAGGAACATCTGTAGACGTAGCTCCCAG
 GGAGCTCAGACAAACACCCCTACCACAGGATGGGCAGTAATATGTGACAGAGCCCAAAGCTGGAAGCAGCATCC
 CTTCCAGCTTAGCTCTTTTCTGACTCTTAGCCACAAACCATCTTAATGTGAGCAACTCTTTTAGGCATTTCTCTTTTCC
 CCGCCTGCACCCACTCTGAACATGCAAAAAGTTGCCAGAGTTGGGGCATTGAGGAAGAGATATTTCTGGAATGTGAGACT
 TGTTATGCCCTCTGTCTCTTTCTCTCCCTCCCCCTCCCCCTCTCCCTCCCCCTCTCCCTCCCATCCCTTTTCTTCCCTTTCA
 CTCTGAAGCAGTTTTAGCTTATTAACAGAAAAACAAACTGGCAAAGCAGGCTTTTTGTGTTAATTGCTCTTTCCCTGATT
 GTGTTTCAGAGAGAAAGGTTATGATTAAATGGGCTCCAGATCTCTTATTGCCCTTATTCTCCACCCCACTTCTTTTAGCA
 AGGTCTGAAAGTTTCAAAGGGAGACCTATAGGTTAATTGTTTAGTTATAGGCAGTGTTAAATTAGGCAGATTGTTGACATA
 TTTATCTTTTTACCCCATCCATCTACCAAACCTGTGTATTTCTTAGTTTGTAGTTTGAAGAGTGGAAAGAGAGAGA
 AGGCCCTCAGACTGATGGGTCTCAGGACGGGTCAAAGGCAAGGCTTTGTGATGTGAGCAAAAGGCAACCAAACCTTAGCC
 TCACTCCACTTTTCTAAAGATGGAATAATCTTTTTTGGGCCCTTGGACTGCTTCTAGGGTAGCATTTTGTAGGTCACTCTTC
 TCCTTTGTACTATTTTGTTCCTGCCCTGATGTCCCTTGGGTCTCCATCCTACTGCCTGGCTTTCTTGGCCCTCATTTCTC
 AGCTTCTGCATTTTCTTCCCTGCTCCTAACAAATGAAGAAGCAGGCTGCAGCCTGCATTGTGGAAGATCTCCAGCCTCTC
 TGTAGGGGATAAGGGGATGTGTAGCATCTGTGTGGATTTTTCACGGACAAGTTCCAGTAGGTGGGACAGTGATGCCGTCAA
 GGCTTAGTTATGATCATGTGTGGTGATAAAGACCATCCACCATACCCCTTTTCCCCTTTGGTTTTGAAGGCCTTGGCCTA
 AGCTACCTGAGGGTTTAGGAGGCTGAACACACACAGTGAGGAGTTAATCTAGGTTGGGAAACTGAGTAAAAGTCAGAG
 GCAGGAATGAGCTGCTGTGGCGTGGGTTTGGAAAGGCTCAGAGGAAAGAACTGCAGGATCAGGGGTGGGAGGGGAGGC
 CCGTGAGGTGCTTCCAGGGAAGAGGGGCTGGGGTTTTAAATAGCATGCTTGGAGGAAGATTTTCCCTCAATTTTCTCTAA
 GTCCTTGAATTACCAAGTAGATTTTTGTAAAACAAATGTAAGTCGATGTTTTCTCTCAATTATCCTAGGAGTGACCTTTA
 TATGTGTGGAAGATTAATGGTATATGCTCCTTATGTCACTGTTTTTGTAGTAAATCCATTTCTTTCTGTGTTTCAGCCT
 ATGACAAAATTGATGTTTACAGGCTGCTTTTTGCTTATAATTGACAACATGTCAAAAAATACCAATTTGTGTCTGTG
 CAGTATGAAGAATTGAGTGAATATTCATTAATGTATTAGCTTGTGTTGCTCTCTGTTTCATATGGCTCTATTCTTAGAA
 ATATAATTTGAATGTGATCTTTCAATAGTCTGAATATTTTACAAATATAGCTATGCTTGTGAAAATACCTCAAAAAG
 AAAAAATCAGCACTGTGTCTTACTTGATATTTCTTGGCCTAGTAATGTACTTGCATTTATGTTCTTAAAGCAGTGTAAG
 TACCAGTAGAATTTCTGTCAAACATCATGATCATTTTAGTACTTTTGTCTTCTCCCATGTGCTTGAAGGAAAAATAAAG
 GTGCACTACCGTATTTCTGTTTTCTATCAAAAAATAAAAAATAATTTAAAAAACAAAAAAAAAAAAAAAAA

human 33B7 (19945) protein

Fig. 27

Rat 1p protein (partial)

LKGARPRVVNSTCSDFNHGSALHIAASNLCLGAACKLLEHGANPALNRKQVPAEVVDPMDMSLDKAEALVAKELRT
 LLEEAVPLSCTLPKVTLPNYDNPVGNLMLSALGLRLGDRVLLDGQKTGTLRFCTTEFASGQWVGVELDEPEGKNDGSVG
 GVRVYFICPPKQGLFASVSKVSKAVDAPPSSVTSTPRTPRMDFSRVTGKGRREHKGKKKSPSSPSLGLSQOREGAKAEVGD
 QVLVAGQNRDCAFLWEDRLCSRLLVWH

Rat 1p DNA (partial, coding:1-804)

CTGAAAGGGGCGAGGCCAGGGTGGTGAACCTCCACCTGCAGTGACTTCAACCATGGCTCAGCTCTGCACATCGCTGCCTC
 GAATCTGTGCCTGGGCGCCGCAATGTTTACTGGAGCATGGTGCCAACCCAGCGCTGAGGAATCGAAAAGGACAGGTAC
 CAGCGGAAGTGGTCCCAGACCCCATGGACATGTCCTTGACAAGGCAGAGGCAGCCCTGGTGGCCAAGGAATTGCGGACG
 CTGCTAGAAGAGGCTGTGCCACTGTCTCTGCACCCCTTCTAAAGTCACTACCCAATATGACAACGTCCCAGGCAATCT
 CATGCTCAGCGCGCTGGGCTGCGTCTAGGAGACCGAGTGCTCCTCGATGGCCAGAAGACGGGCACGCTGAGGTTCTGCG
 GGACCACCGAGTTCCGCAGTGGCCAGTGGGTGGGCGTGGAGCTAGATGAACCGGAAGGCAAGAACGACGGCAGCGTTGGG
 GGTGTCCGGTACTTCATCTGCCCTCCCAAGCAGGGTCTCTTTGCATCTGTGTCCAAGGTCTCCAAGGCAGTGGATGCACC
 CCCCTCATCTGTTACCTCCACGCCCCGCACTCCCCGGATGGACTTCTCCCGTGAACGGGCAAAGGCCGGAGGGAACACA
 AAGGGAAGAAGAAGTCCCCATCTTCCCCATCTCTGGGCAGCCTGCAGCAGCGTGAAGGGGCCAAAGCTGAAGTTGGAGAC
 CAAGTCTTGTGGCAGGCCAGAACAGGGATTGTGCGTTTCTATGGGAAGACAGACTTTGCTCCAGGTTACTGGTATGGCA
 TTGAACGGACAGCCACGGCAAGCATGACGGCTCTGTGTTCCGGTGTCCGGTACTTTACCTGTGCCCCGAGGCACGGG
 GTCTTTGCACCAGCATCTCGTATCCAGAGGATTGGTGGATCCACTGATCCCCCTGGAGACAGTGTGGAGCAAAAAAGT
 GCATCAAGTGACAATGACACAGCCCAAACGCACCTTCACAACAGTCCGGACCCCAAAGGACATTGCATCAGAGAATCTTA
 TCTCCAGGTTACTCTTCTGCTGCTGGTTTCTTGGATGCTGAGGGCGGAGATGCAGTCTTAGAGACCTGGATACCTGACA
 CAGAGACAGAGTCCCCCTTAGCATCTCCTGACACAAGGAGACCCAGTCACCCTAAGATAGAGATTCCAGTGACACCTC
 CAGAATAGAAACCCCGTTAGCCAGCCCTCGATTACTGAGGTCCCATTATTAAACAGATCTCCCATGACGACTCCCCCAAAT
 ACAGACCTCATGTTACCCCAAAGAGATTCCCTGAGTAGCACCTTCAGGCTAGTCCCTGTCCCTACCCCTCAGAGCAGA
 TTTCCCCCAATAAAATTTTCCACATCACCCAAGGGATGCTGACCCTCTCCACGACAGGACGTTCTTGAGTTACAGTGG
 ATTAGAGTCCCATGAATGAAGACCCCCCACCCCGGTTCTCCTTAAGCATAGGTCATACCTCCAGAATAGCCAGCCACA
 TCACTATCCCCATGTAACATCAGTCTCCTCAAAATGGCGTGAGGTCACTAGAAAGACCTTATACTCTCCTCTCCTTCTCA
 GAGATGCCCTCCATTCACTTAAGTCCCTGTTCTACCCCTGAACAAGACACCTAATTAACCGGCCCACTCACCTCAATTA
 CAAACACCAAAATCGTCTGGAAGCATGAATTACAGGACAGCAAGTCTTCCCTGCCCTCTGCACCCCTTGAGAAACCCCAAG
 TGCCCTTGATGAAGCCACCCACATGGCCACAGTCCCTGTGCTGGCCAAGGCTCCAGAAAATTCCTATTTTTTTAAA
 GTAATAACTTCCCCCCTTTGGGGGGATCCCCAAATTTGGAGACCCATTCTAGAACACTGGGGAGTTCAAATTCAGAG
 AGAATATATATATATATAATCCCCAATTCCCCATGCTTCCAAGCCCTACAATCTCTAGAAGACCCCAAATTTCTAATTC
 CCAGGACTTCCCCTACCCAAGTCACAGAATCTTCAAATCCCCAGGGAATCCCAAACCTTAAGATACCAATCCCAAACCCCTC
 AGGAAATCCCCCAACACAAGGTCCCTTAGGACCGGGAGGAAGGAACCTGTTGCCAGGAGAACATCCAGGCTCTCAGGGCA
 TCTCAAACCTGACTCCAGGCACCAGGAGACCCCAAACAGAAAGTCCCATCTTTGGAACAAGGATAGGACTCTAATACCC
 TTAGTCCATGGATCTTTAATTTCCCAACCTCCAAACTCCATGGGCCCAACCTCAAGGGAACCCCAAGATCCAAATCTC
 TGATAACTAATATGTGCAGGGCCCCAGGGCTCTAACAGGACCCCAAATCATGGAGTCCCTACTTCAATCTACCTTCTGGT
 CACAGGTCCAAGACACTAAATCTGAGTCATTGGCCCCAAAGGACTTCACAGCACCTGGGCCAGACTAACAGCCTGAGGGA
 GAACCTGAGGGCCCCGTGGGTCCAGAGCAGACCTGGGGCCCTGACCACCAAGGACAGCTCACGACTGCCCTTCACTGCA
 TGTCCTTAAACTCAGCATGACTCCTGTCTCTTCAATAAAGACGTTTCTATGGCAAAAAAAAAAAAAAAAAAAAAA
 AAA

Fig. 28

Rat 7s Protein (partial)

ADSTSRWAEALREISGRLEMPADSGYPAYLGARLASFYERAGRVKCLGNPEREGSVSIVGAVSPFPGGDFSDPVTSATLG
 IVQVFWGLDKKLAQRKHFPSVNWLISSYKYMRLDEYYDKHFTEFVPLRTRKAKEILQEEEDLAEIVQLVGKASLAETDKI
 TLEVAKLIKDDFLQONGYTPYDRFCPFYKTVGMLSNMISFYDMARRAVETTAQSDNKITWSIIREHMGELIYKLSMKFK
 DPVKDGEAKIKADYAQLLEDMQNAFRSLED

Rat 7s DNA (partial, coding: 1-813)

GCTGACTCTACCTCTAGATGGGCTGAGGCCCTCAGAGAAATCTCTGGTCGCTTAGCTGAAATGCCTGCAGATAGTGGATA
 CCCTGCATACCTTGGTGCCCGACTGGCTTCTTTCTATGAGCGAGCAGGCAGAGTGAAATGTCTTGGAAACCTTGAGAGAG
 AAGGGAGTGTGACGATTGTAGGAGCAGTTTCTCCACCTGGTGGTGATTTTTCTGATCCAGTCACATCTGCTACTCTGGGT
 ATTGTTTCAGGTGTTCTGGGGCTTGGATAAGAAGCTAGCTCAGCGCAAGCACTTCCCGTCCGTCAACTGGCTCATTAGCTA
 CAGCAAGTACATGCGCGCCCTGGACGAGTACTATGACAAACACTTCACAGAGTTCGTGCCTCTGGAGACCAAAGCTAAGG
 AGATTCTGCAGGAAGAGGAGGATCTGGCGGAAATCGTGCAGCTCGTGGGAAAGGCGTCTTTAGCAGAGACAGATAAAATC
 ACCCTGGAGGTAGCAAAACTTATCAAAGATGACTTCTTACAAACAAATGGGTACACTCCTTATGACAGGTCTGTCCATT
 CTATAAGACGGTGGGGATGCTGTCCAACATGATTTTCTATGATATGGCCCGCCGGGCTGTGGAGACCACCGCCGAGA
 GTGACAATAAGATCACATGGTCCATTATCCGTGAGCACATGGGGGAGATTCTCTATAAACTTTCTCCATGAAATTCAGG
 GATCCAGTGAAGGATGGCGAGGCAAGATCAAGGCCGACTACGCACAGCTTCTTGAAGATATGCAGAACGCATTCCGTAG
 CCTGGAAGATTAGAAGTGTGACTTCTCTCTCTCTTCCGCAGCTCATATGTGTATATTTCTGAATTTCTCATCTCCA
 ACCCTTTGCTTCCATATTGTGCAGCTTTGAGACTAGTGCCTCGTGCCTTCTCGTTTCTTTGCTGTTTCTTTGGTAGGTC
 TTATAAAACACACATTCTGTGCTCCGCTGTCTGAAGGAGCTCTGACCTTTGTCTGAAGTGGTGAATGTAGTGCATATG
 ATACACAGTGTAAACATACACATTGTAACATATACGTTCTGTAACTTGTATGTAAGGTGACTACCCCTTCCCTCTCTCC
 AGTAACTGTAAACAGGACTACTGCATGTGCTCTATTGGGGATGGAAGGCCAGATCTCCATACCGTGGACAGGTACATAA
 GGAACTAGACCACCTTGCAACTTAGTGTTTGTGAGTAACCATTTTGCAGGAAGTATTTCCATTTAAAAACAAAGATT
 AATGTTCCAATTATTTGTAGCTTCCCCAGTATCAATCAGGACTGTTTGTGGCGCACTTGGGAACTATTTTGTCTTCTTAA
 CAGACGTTTGCAAGGCTGAACGTAATAGATAAATCAGTTCCTCTGAAAGTGTGAAAGTAAAAAGAGAGCTAGGTGGTCA
 GACTTAAATTGACATCGTCTTGTTTAAGCATATTTTATTTCACTGAGAGATTTAATATCAAGGACTTTTATATACTCAAT
 TACTAGGAAATCTTTTTTAAAGTACAATTTAAAAATCATTGAAATGTGATCCACATCATAGCCATTTTCTTATATTTA
 GTCAGATGAGCTCAGAGTGGGAGGGTGTGGGTAGAATACCACAAGGACACGCAGCAGTGCCTGCAGGCAGTGTGGCCG
 GGGGCCAGAGCGGCATTGTTTTACGAGGTACGTGTGTGGCGTGTGTGTTTGCTTGTGACACTCTGAAAAACAGCAAGCT
 TACCAGTTCCAGGAAATATTTGTTTTCTTCACTGGCTCAGAAAGCTCCTCAAAGTACCTGGTCCCTGAAGCTTCTCTAT
 CTGTTAATAGAGACGAGAGAGGTTCTTAAATTTAACTGGTGACAAAACAAAAGAAAAAAGATCGATTTTTGTCTTGC
 TGTTTTGGTGTGTTTAAATAATAATTCCATATTTGCATAACGAGGCTCGCTTCTGAGAGCTTGGAGATCGTGCTCCCTCT
 TCACTCTCCGGGGTGATAATGCTGGCGCCATGCTACCTCTTCAAGAGGGGAAGGGGATTGAACATGGCTAACACTCTCAA
 GTACACAAGCGTAACGACAAAGTATTTATTTAAGCCTTGGTATGTTGTTTAAATTATTAGGTGGTGCATTTCTTATGGT
 CTTTTGGGTAGACATAGTATACACTTCAGATGTAATGTGTAAATCCTTGCTAGTGCATGTCTACACGATAGACTGCTATT
 CAAGAAGGATATTCTTCCACATAACAATTTAAAACTATTAAATCAGATATGGATTATGCAATGACTTGTGAGAGGTGG
 ATTAACGGTGTGCTTAATCAGTTTGCTTCCAATATGGCTTCGTATCCAGAAGCCCTGACTAGTGGAGATGAGAAAGATT
 TCAAAACCTGTCTGCCTACACCTACCAGCAACCTAGGCTTGTGATCAGAATGAATGATCCCAAGAACTACTTGACCAAG
 TGTGTTTTGTGTGCTTGGATTGAGATGTGCGTCTTCTCCCTCTGAGACTGTTGATGTATGAGTGTGAAGAAGTTACA
 GAAACAACGCTCAGATTTTACGGTAACTTTCCCTCTGCCCACACTGTAGAGTTTCAAGATTGTTCACTGATAGTGCTTCT
 TTCGTAAGGATGTGTTAAATATAGCAGTCTTTTTTAAAGATTATGCAGTCTCTATTTATTGTGCTGTGCCTGGTCCTA
 AGTGCAGCCGGTTAAACAAGTTTCATATGTATTTTCCAGTGTTAAATCTCATACCTATGCCCTTTGGAAGCTCCATCC
 TGAACAATGAATAGAAGAGGCTATATAAATTGCTCCTTATCCTTAAGATTTCACTATCTTTATGTTAAGAGTAATGTAT
 AATTATTAAATCTATGAAAAATAAAAAGTGGATTAAATTAAGAGATC

Fig. 29

Rat 29x protein

ARLPAPPEHARQQPLLSGPEPGSSARVPVPGVASRRQPRGGKPPSGDGLSGPSRPLLHARGEAGLHRQSGRVPHTGTAY
 FADEPTEAQAPGGFCVSPSLLGVRWPACATRTPGSLPLSPPSAQPRTLWPTPPAGPSSRMVARNQVAADNAISPASEPRR
 RPEPSSSSSSSSPAAPARPRPCPVVPAPAPGDTHFRTRSHSDYRRITRTSALLDACGFYWGPLSVHGAHERLRAEPVGT
 FLVRDSRQRNCFFALSVMASGPTSIRVHFQAGRFLDGSRETFDCLFELLEHYVAAPRRMLGAPLRQRRVRPLQELCRQ
 RIVAAGRENLARIPLNPVLRDYLSSFFPFI

Rat 29x DNA (coding: 433-1071)

GCACGGCTCCCCGGCCCCGGAGCATGCGCGACAGCAGCCCCCTCCTCtCCGGCCCTGAGCCCCGGATCGTCCGCCCCGGTTCC
 AGTTCCCCGGCGTGGCCAGTAGGCGGCAGCCGCGAGGCGGCAAGCCACCCAGCGGGACGGCCTGGAGTCGGGCCCCCTCTC
 CACGCCCCCTTCTCCACGCGCGCGGGGAGGCAGGGCTCCACCGCCAGTCTGGAAGGGTCCACATACAGGAACGGCCTAC
 TTCGACAGATGAGCCACCGAGGCTCAGGCTCCGGGCGGATTCTGCGTGTACCCCTCGCTCCTTGGGGTCCGCTGGCCGGC
 CTGTGCCACCCGGACGCCCCGGCTCACTGCCTCTGTCTCCCCATCAGCGCAGCCCCGGACGCTATGGCCCACCCCTCCAG
 CTGGCCCCCTCGAGTAGGATGGTAGCACGTAACCAGGTGGCAGCCGACAATGCGATCTCCCCGGCATCAGAGCCCCGACGG
 CGGCCAGAGCCATCCTCGTCTCTGTCTTCGTCTCGCCGGCGGGCCCCGGCGCGTCCCCGGCCCTGCCCCGGTGGTCCCCGGC
 CCGGGCTCCGGGCGACACTCACTTCCGCACCTTCCGCTCCCACTCTGATTACCGGCGCATCACGCGGACCAGCGCTCTCC
 TGGACGCCTGCGGCTTCTACTGGGGACCCCTGAGCGTGCATGGGGCGCACGAACGGCTGCGTGCCGAGCCCGTGGGCACC
 TTCTTGGTGCGCGACAGTCGCCAGCGGAAGTCTTCTTCGCGCTCAGCGTGAAGATGGCTTCGGGCCCCACGAGCATTCG
 TGTGCACTTCCAGGCCGGCCGCTTCCACCTGGACGGCAGCCGCGAGACCTTCGACTGCCTCTTCGAGCTGCTGGAGCACT
 ACGTGGCGGGCGCCGCGCCGCATGTTGGGGGCCCCACTGCGCCAGCGCCGCGTGCAGGAGCTGTGTCGCCAG
 CGCATCGTGGCCGCGGTGGGTGCGGAGAACCTGGCAGCATCCCTCTTAACCCGGTACTCCGTGACTACCTGAGTTCCTT
 CCCCTTCCAGATCTGACCGGCTGCCGCCGTGCCCCGAGCATTAAGTGGGAGCGCCTTATTATTTCTTATTATTAATTATT
 ATTATTTTTTcTGAACCACGTGGGAGCCCTCCCCGCCTAGGTGGAGGGAGTGGGTGTGGAGGGTGAAGTGCCTCCCACT
 TCTGGCTGGAGACCTTATCCCGCTCTCGGGGGGCTCCCTCCTGGTGTCTCCCTCCCGGTCCCCCTGGTTGTAGCAGCT
 TGTGTCTGGGGCCAGGACCTGAAGTCCACGCCTACCTCTCCATGTTTACATGTTCCAGTATCTTTGCACAAACCAGGGG
 TGGGGGAGGGTCTCTGGCTTCATTTTTCTGCTGTGCAGAATATTCTATTTTATATTTTACATCCAGTTTAGATAATAAAA
 CTTTATTATGAAAGTTTTTTTTTTTAAAGAAAAAAAAAAAAAAAAAAAAA

Fig. 30

Rat 25r DNA (coding 130-

GGCACGGCTCCCGGCCCCGGAGCATGCGCGACAGCAGCCCCGGAACCCCCAGCCGGCGGCGCCCGCGTCCCGCCGCCAGC
GCAGCCCCGGACGCTATGGCCACCCCTCCAGCTGGCCCCCTCGAGTAGGATGGTAGCACGTAACCAGGTGGCAGCCGACA
ATGCGATCTCCCGGCATCAGAGCCCCGACGGCGGCCAGAGCCATCCTCGTCCTCGTCTTCGTCTCGCCGGCGGCCCCG
GCGCGTCCCGGCCCCGCGGTGGTCCCGGCCCCGGCTCCGGGCGACACTCACTTCCGCACCTTCCGCTCCCACTCTGA
TTACCGGCGCATCACGCGGACCAGCGCTCTCCTGGACGCCTGCGGCTTCTACTGGGGACCCCTGAGCGTGCATGGGGCGC
ACGAACGGCTGCGTGCCGAGCCCGTGGGCACCTTCTTGGTGCGCGACAGTCGCCAGCGGAACCTGCTTCTTCGCGCTCAGC
GTGAAGATGGCTTCGGGCCCCACGAGCATTCGTGTGCACTTCCAGGCCGGCCGCTTCCACCTGGACGGCAGCCGCGAGAC
CTTCGACTGCCTCTTCGAGCTGCTGGAGCACTACGTGGCGGCGCCGCGCCGATGTTGGGGGCCCCACTGCGCCAGCGCC
GCGTGCGGCGCTGCAGGAGCTGTGTCGCCAGCGCATCGTGGCCGCGGTGGGTGCGGAGAACCTGGCACGCATCCCTCTT
AACCCGGTACTCCGTGACTACCTGAGTTCCTTCCCTTCCAGATCTGACCGGCTGCCGCGGTGCCCGCAGCATTAAGTGG
GAGCGCCTTATTATTTCTTATTATTAATTATTATTTTCTGGAACACGTGGGAGCCCTCCCCGCTAGGTGGGAGG
GAGTGGGTGTGGAGGGTGAGATGCCTCCCACTTCTGGCTGGAGACCTTATCCCGCTCTCGGGGGGCTCCCTCCTGGT
GCTCCCTCCCGGTCCCCCTGGTTGTAGCAGCTTGTGTCTGGGGCCAGGACCTGAACTCCACGCTACCTCTCCATGTTTA
CATGTTCCAGTATCTTTGCACAAACCAGGGGTGGGGGAGGGTCTCTGGCTTCATTTTTCTGCTGTGCAGAATATTCTAT
TTTATATTTTACATCCAGTTTAGATAATAAACTTTATTATGAAAGTTTTTTTTTAAAAA

Fig. 31

Rat 5p protein

MPSQMEHAMETMMLTFHRFAGEKNYLTKEDLRVLMEREPGFLENQKDPLAVDKIMKDLQCRDGKVGFSFLSLVAGLI
IACNDYFVVHMKQKK

Rat 5p DNA (coding: 52-339)

CTTCCAAAGACTGCAGCGCCTCAGGGCCCAGGTTTCAACAGATTCTTCAAAATGCCATCCCAAATGGAGCATGCCATGGA
AACCATGATGCTTACATTTACAGGTTTGCAGGGGAAAAAACTACTTGACAAAGGAGGACCTGAGAGTGCTCATGGAAA
GGGAGTTCCCTGGGTTTTTGGAAAATCAAAAGGACCTCTGGCTGTGGACAAAATAATGAAAGACCTGGACCAGTGCCGA
GATGGAAAAGTGGGCTTCCAGAGCTTTCTATCACTAGTGGCGGGGCTCATCATTGCATGCAATGACTATTTTGTAGTACA
CATGAAGCAGAAGAAGTAGGCCAACTGGAGCCCTGGTACCCACACCTTGATGCGTCCTCTCCCATGGGGTCAACTGAGGA
ATCTGCCCCACTGCTTCCTGTGAGCAGATCAGGACCTTAGGAAATGTGCAAATAACATCCAACTCCAATTCGACAAGCA
GAGAAAGAAAAGTTAATCCAATGACAGAGGAGCTTTCGAGTTTTATATTGTTGCATCCGGTTGCCCTCAATAAAGAAAG
TCTTTTTTTTTTAAGTTCCGAAAAAAAAAAAAAAAAAAAAA

Fig. 32

Rat 7q protein

MAYAYLFKYIIIIGDTGVGKSCLLQLFTDKRFQPVHDLTIGVEFGARMITIDGKQIKLQIWDTAGQESFRSITRSYYRGAA
GALLVYDITRRDTFNHLTTWLEDARQHSNSNMVIMLIGNKSDLESRRREVKKEEGEAFAREHGLIFMETSAKTASNVEEAF
INTAKEIYEKIQEGVFDINNEANGIKIGPQHAATNASHGGNQGGQQAGGGCC

Rat 7q DNA (coding 1-639)

ATGGCGTACGCCTATCTCTTCAAGTACATCATCATCGGCGACACAGGTGTTGGTAAATCGTGCTTATTGCTACAGTTTAC
AGACAAGAGGTTTCAGCCGGTGCATGACCTCACAATTGGTGTAGAGTTTGGTGTCTCGAATGATAACCATTGATGGGAAAC
AGATAAACTCCAGATCTGGGATACAGCAGGGCAGGAGTCCTTTTCGTTCTATCACAAGGTCATATTACAGAGGTGCAGCG
GGGGCTTTACTAGTGTATGATATTACAAGGAGAGACAGTTCAACCACTTGACAACCTGGTTAGAAGACGCCCCTCAGCA
TTCCAATTCCAACATGGTCATCATGCTTATTGGAAATAAAAGTGACTTAGAATCTAGGAGAGAACTGAAAAAGGAAGAAG
GTGAAGCTTTTGCACGAGAGCATGGACTTATCTTCATGGAACTTCTGCCAAGACTGCTTCTAATGTAGAGGAGGCATTT
ATTAACACAGCAAAAGAAATTTATGAAAAAATCCAAGAAGGGGTCTTTGACATTAATAATGAGGCAAAACGGCATCAAAAT
TGGCCCTCAGCATGCTGCTACCAATGCATCTCACGGAGGCAACCAAGGAGGCAGCAGGCAGGGGAGGCTGCTGCTGA

Fig. 33

Rat 19r protein

MVLLKEYRVILPVSVD EYQVGQLYSVAEASKNETGGGEGVEVLVNEPYEKDDGEKGQYTHKIYHLQSKVPTFVRMLAPEG
 ALNIHEKAWNAYPYCRTVITNEYMKEDFLIKIETWHKPD LGTQENVHKLEPEAWKHVEAIYIDIADRSQVLSKDYKAEED
 PAKFKSIKTGRGPLGPNWKQELVNQKDCPYMCAYKLVTVKFWWGLQNKVENFIHKQEKRLFTNFHRQLFCWLDKWVDLT
 MDDIRRMEEETKRQLDEM RQKDPVKGMTADD

Rat 19r DNA (coding 1-816)

ATGGTGCTGCTCAAGGAATATCGGGTCATCCTGCCTGTGTCTGTAGATGAGTATCAAGTGGGGCAGCTGTACTCTGTGGC
 TGAAGCCAGTAAAAATGAACTGGTGGTGGGGAAGGTGTGGAGGTCCTGGTGAACGAGCCCTACGAGAAGGATGATGGCG
 AGAAAGGCCAGTACACACACAAGATCTACCCTTACAGAGCAAAGTTCACGTTTGTTCGAATGCTGGCCCCAGAAGGC
 GCCCTGAATATACATGAGAAAGCCTGGAATGCCTACCCTTACTGCAGAACCGTTATTACAAATGAGTACATGAAGGAAGA
 CTTTCTCATTTAAATTTGAAACCTGGCACAAGCCAGACCTTGGCACCCAGGAGAATGTGCATAAACTGGAGCCTGAGGCAT
 GGAAACATGTGGAAGCTATATATATAGACATCGCTGATCGAAGCCAAGTACTTAGCAAGGATTACAAGGCAGAGGAAGAC
 CCAGCAAAATTTAAATCTATCAAAACAGGACGAGGACCATTGGGCCCGAATTGGAAGCAAGAACTTGTCAATCAGAAGGA
 CTGCCCATATATGTGTGCATACAACTGGTTACTGTCAAGTTCAAGTGGTGGGGCTTGCAGAACAAAGTGGAAAACTTTA
 TACATAAGCAAGAGAAGCGTCTGTTTACAACTTTACAGGCAGCTGTTCTGTTGGCTTGATAAATGGGTTGATCTGACT
 ATGGATGACATTTCGGAGGATGGAAGAAGAGACGAAGAGACAGCTGGATGAGATGAGACAAAAGGACCCCGTGAAAGGAAT
 GACAGCAGATGACTAG

Fig. 34

Monkey KChIP4c (jlkxa053c02) DNA sequence (CD: 122-811)

CGCTCTCCTCCTCCCTTTCTCTAGCAGTAGCCTTCTTAATGTAGTTTAATGGCTTTACAAAGAAAGCCAGGCAGAGGAG
 CACTTCTCAGTGGCTGTGGTCGGACCATGACCTAGCTGACCATGAAC TTGGAAGGGCTTGAAATGATAGCAGTTCTGATC
 GTCATTGTGCTTTTTTGTAAATTATTGGAACAGTTTGGGCTGATTGAAGCAGGTTTAGAAGACAGCGTGGAAGATGAACT
 GGAGATGGCCACTGTCAGGCATCGGCCTGAGGCCCTTGAGCTTCTGGAAGCCCAGAGCAAATTTACCAAGAAAGAGCTTC
 AGATCCTTTACAGAGGATTTAAGAACGAATGCCCCAGTGGTGTGTTAATGAAGAAACCTTCAAAGAGATTTACTCGCAG
 TTCTTTCCACAGGGAGACTCTACAACATATGCACATTTTCTGTTCAATGCGTTTGATACGGACCACAATGGAGCTGTGAG
 TTTTCGAGGATTTTCATCAAAGGTCTTCCATTTTGTCTCCGGGGACAGTACAAGAAAACTCAATTGGGCATTTAATCTGT
 ATGATATAAAATAAGATGGCTACATCACTAAAGAGGAAATGCTTGATATAATGAAAGCAATATACGACATGATGGGTAAA
 TGTACATATCCTGTCTCAAAGAAGATGCACCCAGACAACACGTCGAAACATTTTTTCAGAAAATGGACAAAAATAAAGA
 TGGGGTTGTTACCATAGATGAGTTCATTGAAAGCTGCCAAAAAGATGAAAACATAATGCGCTCCATGCAGCTCTTTGAAA
 ATGTGATTTAACTTGTCAACTAGATCCTGAATCCAACAGACAAATGTGAACATTTCTACCACCCTTAAAGTCGGAGCTAC
 CACTTTTAGCATAGATTGCTCAGCTTGACACTGAAGCATATTATGCAAACAAGCTTTGTTTTAATATAAGCAATCCCCA
 AAAGATTTGAGTTTCTCAGTTATAAATTTGCATCCTTTCCATAATGCCACTGAGTTCATGGGATGTTCTAACTCATTTC
 TACTCTGTGAATATTCAAAGTAATAGAATCTGGCATATAGTTTATTGATTCCCTAGCCATGGGATTATTGAGGCTTTC
 ACATATCAGTGATTTTAAATAACAGTGTTTTTTGCTACTCATTTGTATGTATTCAGTCTTAGGATTTTGAATGGTTTTTC
 TAATATACTGACATCTGCATTTAATTTCCAGAAATTAATTAATTTTCATGTCTGAATGCTGTAATTCATTATATACT
 TTAAGTAAACAAATAAGATTACTACAATTAACACATAGTTCAGTTTCTATGGCCTTCACTTCCCACCTTCTATTAGAA
 ATTAATTTTATCTGGTATTTTTTAAACATTTAAAAATTTATCATCAGATATCAGCATATGCCTAATTATGCCTAATGAAAC
 TTAATAAGCATTTAATTTTCCATCATACATTATAGTCAAGGCCATATACTATATATAATTTTGGATTTGTTTAATCTTA
 CAGGCTGTTTTCCATTGTATCATCAAGTGGAAGTTCAAGACGGCATCAAACAAAACAAGGATGTTTACAGACATATGCAA
 AGGGTCAGGATATCTATCCTCCAGTATATGTTAATGCTTAATAACAAGTAATCCTAACAGCATTAAGGCCAAATCTGTC
 CTCTTTCCCCTGACTTCTTACAGCATGTTTATATTACAAGCCATTGAGGACAAAGAAACCTTGACTACCCCACTGTCT
 ACTAGGAACAAACAAACAGCAAGCAAAATTCACTTTGAAAGCACCAGTGGTTCATTACATTGACAACACTACTACCAAGAT
 TCAGTAGAAAATAAGTGCTCAACAATAATCCAGATTACAATATGATTTAGTGCATCATAAAATTTCAACAATTCAGATT
 ATTTTAAATCACCTCAGCCACAACGTAAAGTTGCCACATTACTAAAGACACACACATCGTCCCTGTTTTGTAGAAATAT
 CACAAAGACCAAGAGGCTACAGAAGGAGGAAATTTGCAACTGTCTTTGCAACAATAAATCAGGTATCTATTCTGGTGTAG
 AGATAGGATGTTGAAAGCTGCCCTGCTATCACCAGTGTAGAAATTAAGAGTAGTACAATACATGTACACTGAAATTTGCC
 ATCGCGTGTGTTGTGTAACTCAATGTGCACATTTTGTATTTCAAAAAGAAAAATAAAAGCAAAATAAAATGTTTATAAC
 TCTAAAAAAAAAAAAAAAAAAAAA

Monkey KChIP4c protein sequence

MNLEGLEMI AVLIVIVLFVKLLLEQFGLIEAGLEDSVEDELEMATV RHRPEALELLEAQSKFTKKELQILYRGFKNECPSG
 VVNEETFKEIYSQFFPQGDSTTYAHFLFNAFDTDHNGAVSFEDFIKGLSILLRGT VQEKLNWAFNLYDINKDGYITKEEM
 LDIMKAIYDMMGKCTYPVLKEDAPRQHVETFFQKMDKNKDG VVTIDEFIESCQK DENIMRSMQLFENVI.

Fig. 35

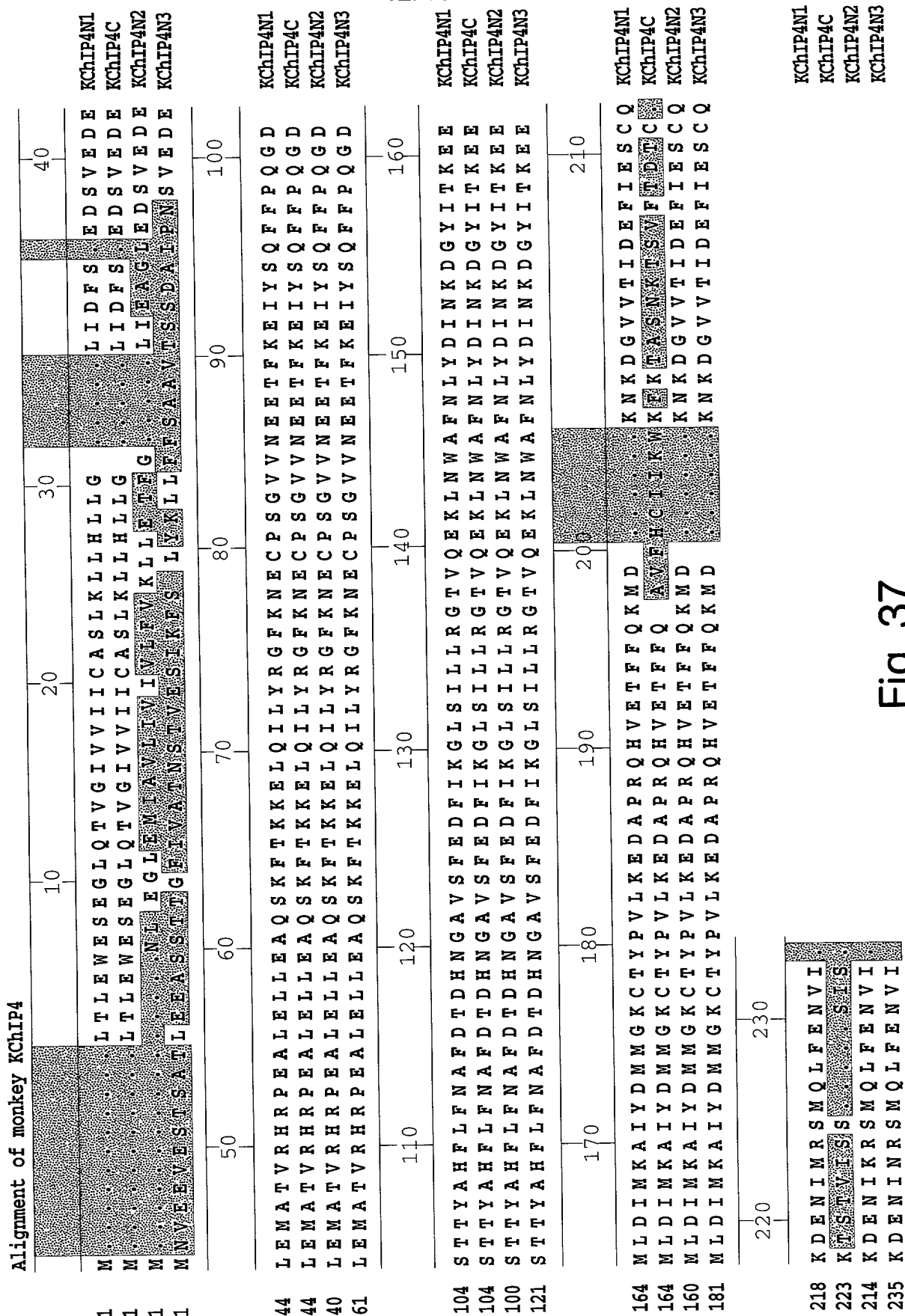
Monkey KChIP4d (j1kx015b10) DNA sequence (CD:64-816)

GTGCACAGACGCCCCCTGGCCGGTGGACTCCTGAGTCTTACTCCTGCACCCTGCGTCCCCAGACATGAATGTGAGGAGAGT
 GGAAAGCATTTTCGGCTCAGCTGGAGGAGGCCAGCTCCACAGGCGGTTTCCTGTATGCTCAGAACAGCACCAGCGCAGCA
 TTAAAGAGCGGCTCATGAAGCTCTTGCCCTGCTCAGCTGCCAAAACATCGTCTCCTGCTATTCAAACAGCGTGGAAGAT
 GAACTGGAGATGGCCACTGTGAGGCATCGGCCTGAGGCCCTTGAGCTTCTGGAAGCCCAGAGCAAATTTACCAAGAAAGA
 GCTTCAGATCCTTTACAGAGGATTTAAGAACGAATGCCCCAGTGGTGTGTTAATGAAGAAACCTTCAAAGAGATTTACT
 CGCAGTTCTTTCCACAGGGAGACTCTACAACATATGCACATTTTCTGTTCAATGCGTTTGATACGGACCACAATGGAGCT
 GTGAGTTTCGAGGATTTTCATCAAAGGTCTTTCCATTTTGCTCCGGGGGACAGTACAAGAAAACTCAATTGGGCATTTAA
 TCTGTATGATATAAATAAAGATGGCTACATCACTAAAGAGGAAATGCTTGATATAATGAAAGCAATATACGACATGATGG
 GTAATGTACATATCCTGTCTCAAAGAAGATGCACCCAGACAACCGTCGAAACATTTTTTCAGAAAATGGACAAAAAT
 AAAGATGGGGTTGTTACCATAGATGAGTTCATTGAAAGCTGCCAAAAAGATGAAAACATAATGCGCTCCATGCAGCTCTT
 TGAAAATGTGATTTAACTTGTCAACTAGATCCTGAATCCAACAGACAAATGTGAACATTTCTACCACCTTAAAGTCGGA
 GCTACCACCTTTTAGCATAGATTGCTCAGCTTGACACTGAAGCATATTATGCAACAAGCTTTGTTTTAATATAAAGCAAT
 CCCCAAAAGATTTGAGTTTCTCAGTTATAAATTTGCATCCTTTCCATAATGCCACTGAGTTCATGGGATGTTCTGACTCA
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 CTTTCACATATCAGTGATTTTAAAATACCAGTGTTTTTTTGCTACTCATTTGTATGTATTGAGTCCCTAGGATTTTGAATGG
 TTTTCTAATATACTGACATCTGCATTTAATTTCCAGAAATTAATTAATTTTCATGTCTGAATGCTGTAATTCATTTAT
 ATACTTTAAGTAAACAAATAAGATTACTACAATTAACACATAGTTCAGTTTCTATGGCCTTCACTTCCCACCTTCTAT
 TAGAAATTAATTTTATCTGGTATTTTTTAAACATTTAAAAATTTATCATCAGATATCAGCATATGCCTAATTATGCCTAAT
 GAAACTTAATAAGCATTTAATTTCCATCATACTATAGTCAAGGCCTATATACTATATATAATTTTGATTGTTTAA
 TCTTACAGGCTGTTTTCCATTGTATCATCAAGTGGAGTTCAAGACGGCATCAAACAAAACAAGGATGTTTACAGACATA
 TGCAAGGGTCAGGATATCTATCCTCCAGTATATGTTAATGCTTAATAACAAGTAATCCTAACAGCATTAAGGCCAAAT
 CTGTCTCTTTCCCTGACTTCCTTACAGCATGTTTTATTAACAAGCCATTCAGGGACAAAGAAACCTTGACTACCCAC
 TGTCTACTAGGAACAAACAAACAGCAAGCAAAATTCATTTGAAAGCACCAGTGGTTCCATTACATTGACAACACTACC
 AAGATTTCAGTAGAAAATAAGTGCTCAACAATAATCCAGATTACAATATGATTTAGTGCATCATAAAATTCACAACATTC
 AGATTATTTTAAATCACCTCAGCCACAACGTGTAAGTTGCCACATTACTAAAGACACACACATCGTCCCTGTTTTGTAGA
 AATATCACAAAGACCAAGAGGCTACAGAAGGAGGAAATTTGCAACTGTCTTTGCAACAATAAATCAGGTATCTATTCTGG
 TGTAGAGATAGGATGTTGAAAGCTGCCCTGCTATCACCAGTGTAGAAATTAAGAGTAGTACAATACATGTACACTGAAAT
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 AAAAAAAAAAAAAAAAAA

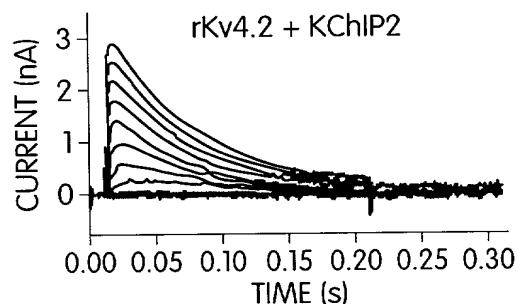
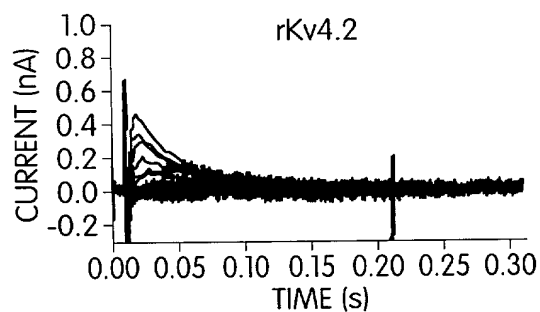
Monkey KChIP4d protein sequence

MNVRRVESISAQLEEASSTGGFLYAQNSTKRSIKERLMKLLPCSAAKTSSPAIQNSVEDELEMATVRHRPEALELLEAQS
 KFTKKELQILYRGFKNECPSGVVNEETFKEIYSQFFPQGDSTTYAHFLFNAFDTDHNGAVSFEDFIKGLSILLRGTVQEK
 LNWAFNLYDINKDGYITKEEMLDIMKAIYDMMGKCTYPVLKEDAPRQHVETFFQKMDKNKDGVVTTIDEFIESCQKDENIM
 RSMQLFENVI.

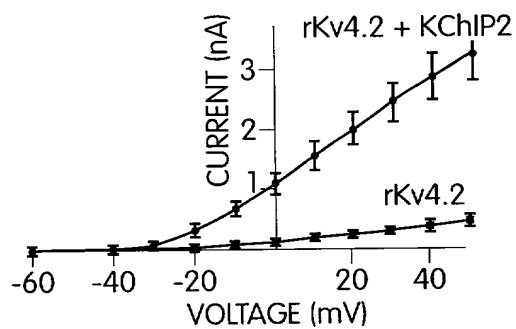
Fig. 36



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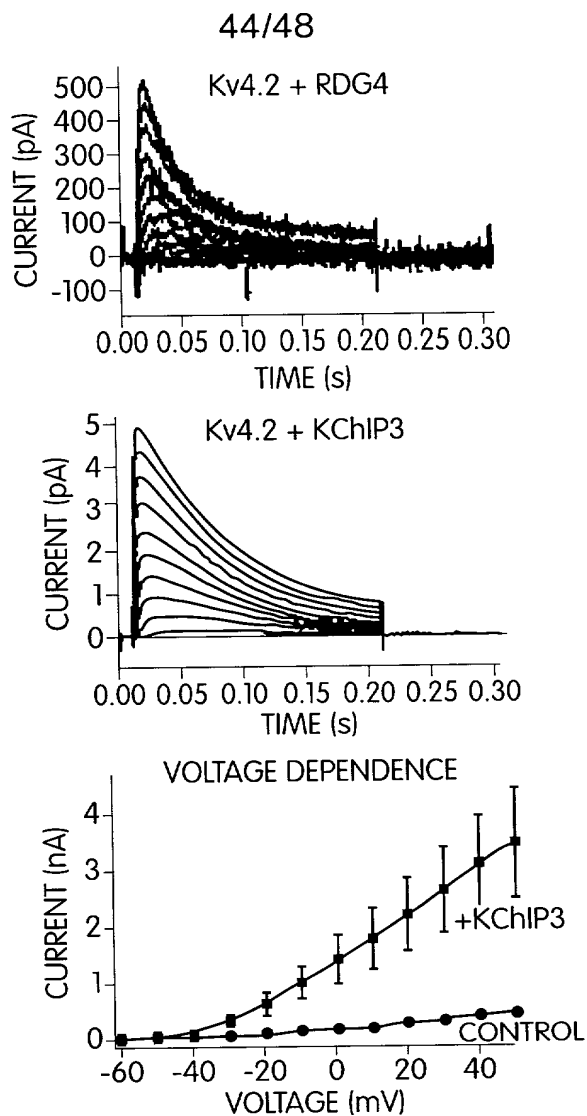


VOLTAGE-DEPENDENCE



CURRENT PARAMETER	CHO	
	rKv4.2	rKv4.2 +KChIP2
PEAK CURRENT (nA/cell, at 50 mV)	0.51 ±0.098	3.3 ±0.45
PEAK CURRENT DENSITY (pA/pF, at 50 mV)	18.6 ±2.8	196.6 ±26.6
INACTIVATION TIME CONSTANT (ms, at 50 mV)	28.47 ±3.5	95.14 ±8.3
RECOVERY FROM INACTIVATION TIME CONSTANT (ms, at -80 mV)	257.9	49.5
ACTIVATION $V_{1/2}$ (mV)	20.5	-2.2
STEADY-STATE INACTIVATION $V_{1/2}$ (mV)	-47.1	-45.7

Fig. 38



CURRENT PARAMETER	CHO	
	rKv4.2 +RBG4	rKv4.2 +KChIP3
PEAK CURRENT (nA/cell, at 50 mV)	0.46 ±0.084	3.5 ±0.99
PEAK CURRENT DENSITY (pA/pF, at 50 mV)	29.7 ±11.2	161.7 ±21.8
INACTIVATION TIME CONSTANT (ms, at 50 mV)	29.5 ±9.5	67.2 ±14.1
RECOVERY FROM INACTIVATION TIME CONSTANT (ms, at -80 mV)	435.9	130.8
ACTIVATION $V_{1/2}$ (mV)	4.1	6.1

Fig. 39

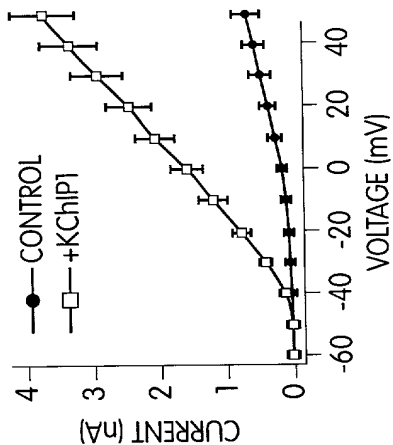


Fig. 40C

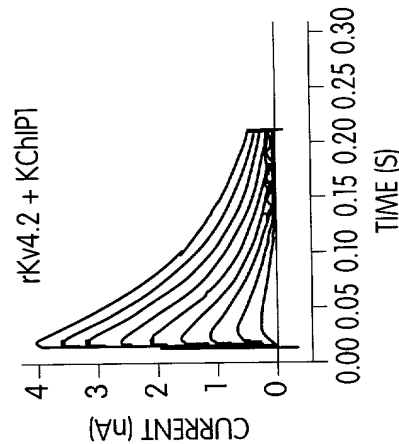


Fig. 40B

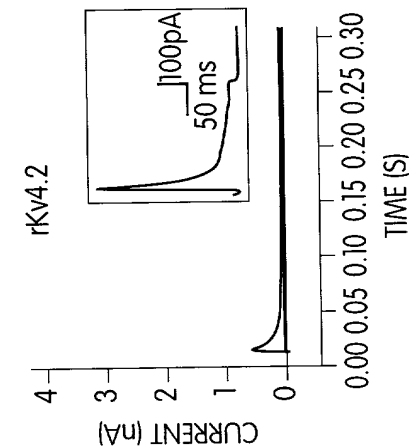


Fig. 40A

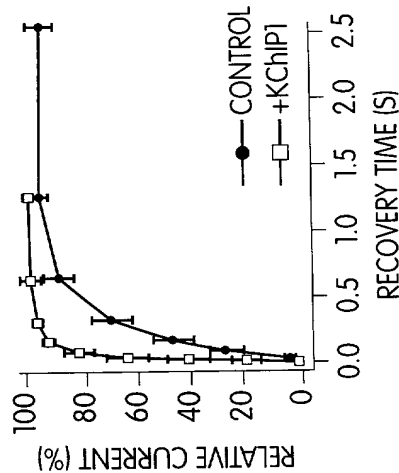


Fig. 40F

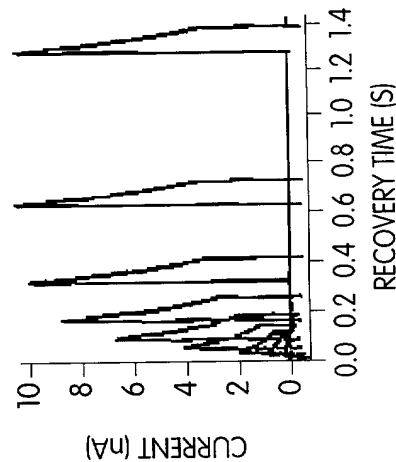


Fig. 40E

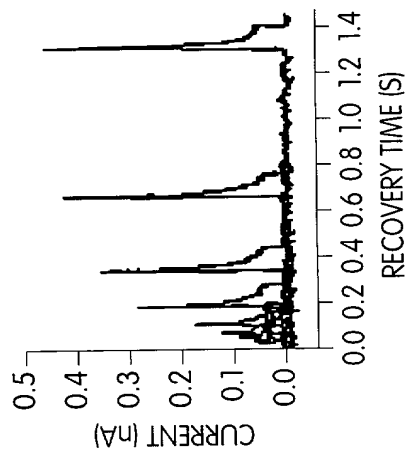


Fig. 40D

Fig. 41

a

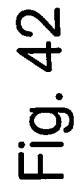


Fig. 42

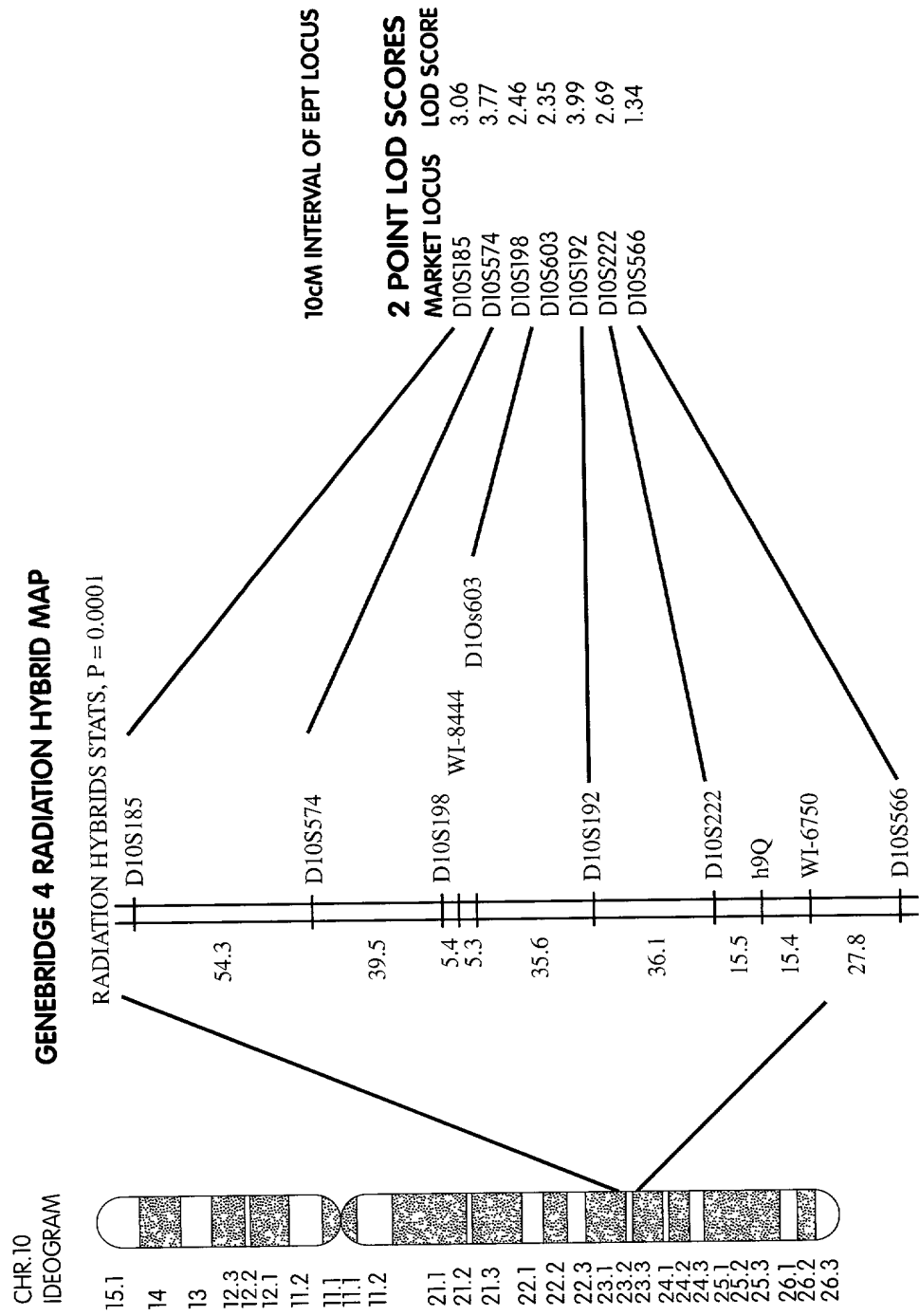


Fig. 43

Attorney's

Docket

Number MNI-070CP4

Declaration, Petition and Power of Attorney

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

POTASSIUM CHANNEL INTERACTORS AND USES THEREFOR

the specification of which

(check one)

is attached hereto.

_____ was filed on _____ as _____

Application Serial No. _____

and was amended on _____
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

This application in part discloses and claims subject matter disclosed in my earlier filed application(s), as follows:

X Serial No.60/110,033, filed November 25, 1998 ;
Serial No.60/109,333, filed November 20, 1998
Serial No.60/110,277, filed November 30, 1998, as to which I claim priority
benefit under Title 35, United States Code, §119(e).

X Serial No.09/298,731, filed April 23, 1999 ;
 Serial No.09/350,614, filed July 9, 1999 ;
 Serial No.09/350,874, filed July 9, 1999 ;
 Serial No.09/399,913, filed September 21, 1999 ;
 Serial No.09/400,492, filed September 21, 1999, as to which I claim priority
 benefit under Title 35, United States Code, §120.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56, including all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application(s) and the national or PCT international filing date of the continuation-in-part application.

AS TO PARENT APPLICATION:

As to the subject matter of this application which is common to said earlier application, I do not know and do not believe that the same was ever known or used in the United States of America before my or our invention thereof or patented or described in any printed publication in any country before my or our invention thereof, or more than one year prior to said earlier application, or in public use or on sale in the United States of America more than one year prior to said earlier application; that the common subject matter has not been patented or made the subject of an inventor's certificate issued before the date of said earlier application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months prior to said earlier application; and

As to applications for patents or inventor's certificate or PCT international application(s) designating at least one country other than the United States of America, on the common subject matter, filed in or designating any country foreign to the United States of America, prior to said earlier application by me or my legal representatives or assigns,

Check one:

☒ no such applications have been filed.

☐ such applications have been filed as follows

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED WITHIN 12 MONTHS
(6 MONTHS FOR DESIGN) PRIOR TO SAID EARLIER U.S. APPLICATION

Country	Application Number	Date of Filing (month,day,year)	Priority Claimed Under 35 USC 119
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ALL FOREIGN APPLICATION(S), IF ANY, FILED MORE THAN 12 MONTHS
(6 MONTHS FOR DESIGN) PRIOR TO SAID EARLIER U.S. APPLICATION

AS TO THIS APPLICATION:

As to the subject matter of this application which is not common to said earlier application, I do not know and do not believe that the same was ever known or used in the United States of America before my or our invention thereof or patented or described in any printed publication in any country before my or our invention thereof, or more than one year prior to this application, or in public use or on sale in the United States of America more than one year prior to this application; that said non-common subject matter has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months prior to this application; and

As to applications for patents or inventor's certificate or PCT international application(s) designating at least one country other than the United States of America, on said non-common subject matter, filed in or designating any country foreign to the United States of America, prior to this application by me or my legal representatives or assigns,

Check one:

☐ no such applications have been filed.

☒ such applications have been filed as follows

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED WITHIN 12 MONTHS
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

Country	Application Number	Date of Filing (month,day,year)	Priority Claimed Under 35 USC 119
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ALL FOREIGN APPLICATION(S), IF ANY, FILED MORE THAN 12 MONTHS
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

CLAIM FOR BENEFIT OF U.S. PROVISIONAL APPLICATION(S)

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

<u>60/110,033</u>	<u>November 25, 1998</u>
(Application Serial No.)	(Filing Date)

<u>60/109,333</u>	<u>November 20, 1998</u>
(Application Serial No.)	(Filing Date)

<u>60/110,277</u>	<u>November 30, 1998</u>
(Application Serial No.)	(Filing Date)

CLAIM FOR BENEFIT OF U.S. PATENT APPLICATION(S)

I hereby claim the benefit under 35 U.S.C. §120 of any United States patent application(s) listed below.

<u>09/298,731</u>	<u>April 23, 1999</u>
(Application Serial No.)	(Filing Date)

<u>09/350,614</u>	<u>July 9, 1999</u>
(Application Serial No.)	(Filing Date)

<u>09/350,874</u>	<u>July 9, 1999</u>
(Application Serial No.)	(Filing Date)

<u>09/399,913</u>	<u>September 21, 1999</u>
(Application Serial No.)	(Filing Date)

<u>09/400,492</u>	<u>September 21, 1999</u>
(Application Serial No.)	(Filing Date)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

James E. Cockfield	Reg. No. 19,162	Nicholas P. Triano III	Reg. No. 36,397
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Ralph A. Loren	Reg. No. 29,325	DeAnn F. Smith	Reg. No. 36,683
Giulio A. DeConti, Jr.	Reg. No. 31,503	William D. DeVaul	Reg. No. 42,483
Ann Lampert Hammitte	Reg. No. 34,858	David J. Ridders	Reg. No. 43,882
Elizabeth A. Hanley	Reg. No. 33,505	Chi Suk Kim	Reg. No. 42,728
Amy E. Mandragouras	Reg. No. 36,207	Maria C. Laccotripe	Limited Recognition
Anthony A. Laurentano	Reg. No. 38,220		Under 37 C.F.R. § 10.9(b)
Jane E. Remillard	Reg. No. 38,872	Debra J. Milasincic	Reg. No. P46,931
Jeremiah Lynch	Reg. No. 17,425	David R. Burns	Reg. No. P46,590
Kevin J. Canning	Reg. No. 35,470	Sean D. Detweiler	Reg. No. 42,482
Jeanne M. DiGiorgio	Reg. No. 41,710	Peter S. Stecher	Reg. No. P47,259
Megan E. Williams	Reg. No. 43,270		

all of: LAHIVE & COCKFIELD, LLP, 28 State Street, Boston, MA 02109

and to: Jean M. Silveri	Reg. No. 39,030	Mark F. Boshar	Reg. No. 35,456
Theodore Allen	Reg. No. 41,578	Scott A. Brown	Reg. No. 32,724
Jill E. Uhl	Reg. No. 43,213		

of: Millennium Pharmaceuticals, Inc., 75 Sidney Street, Cambridge, MA 02139

Send Correspondence to Amy E. Mandragouras at **Customer Number 000959** whose address is:

Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109

Direct Telephone Calls to: (name and telephone number)

Amy E. Mandragouras, (617) 227-7400

Wherefore I petition that letters patent be granted to me for the invention or discovery described and claimed in the attached specification and claims, and hereby subscribe my name to said specification and claims and to the foregoing declaration, power of attorney, and this petition.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Inventor's signature	Date
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Citizenship U.S.	
Post Office Address (if different)	

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Inventor's signature	Date
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Inventor's signature	Date
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Citizenship U.S.	
Post Office Address (if different)	

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: Kenneth Rhodes *et al.*

Serial No.: N/A

Filed: Herewith

For: *POTASSIUM CHANNEL INTERACTORS AND
USES THEREFOR*

Attorney Docket No.: MNI-070CP4

Assistant Commissioner for Patents
Box Sequence
Washington, D.C. 20231

TRANSMITTAL LETTER FOR DISKETTE CONTAINING SEQUENCE LISTING

Dear Sir:

Enclosed is a diskette which contains a computer readable form of the Sequence Listing for the patent application filed herewith. The Sequence Listing complies with the requirements of 37 C.F.R. § 1.821. The material on this diskette is identical in substance to the sequence listing appearing on pages 1-92 of the Sequence Listing which is submitted herewith, as required by 37 C.F.R. § 1.821(f). The computer readable form of the Sequence Listing contained on the enclosed diskette is understood to comply with the requirements of § 1.824(d).

"Express Mail" mailing label number EL 011 360 044 US

Date of Deposit September 27, 2000

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Box Sequence, Washington, D.C. 20231

Signature

Nelson Barros

Nelson F. Barros

Please Print Name of Person Signing

LAHIVE & COCKFIELD, LLP
Attorneys at Law

By

Laccotripe
Maria C. Laccotripe, Ph.D.

Agent for Applicants

Limited Recognition Under 37 C.F.R. §10.9(b)

28 State Street

Boston, MA 02109

Telephone: 617-227-7400

Facsimile: 617-742-4214

SEQUENCE LISTING

<110> Rhodes, Kenneth
Betty, Maria
Ling, Huai-Ping
An, Wenqian

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Gln Leu Phe Gln Asn Val Met
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Thr	Lys 50	Arg	Glu	Leu	Gln	Val 55	Leu	Tyr	Arg	Gly	Phe 60	Lys	Asn	Glu	Cys
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Thr	Ala	Leu 115	Ser	Ile	Leu	Leu	Arg 120	Gly	Thr	Val	His	Glu 125	Lys	Leu	Arg
Trp	Thr 130	Phe	Asn	Leu	Tyr	Asp 135	Ile	Asn	Lys	Asp	Gly 140	Tyr	Ile	Asn	Lys
Glu 145	Glu	Met	Met	Asp	Ile 150	Val	Lys	Ala	Ile	Tyr 155	Asp	Met	Met	Gly	Lys 160
Tyr	Thr	Tyr	Pro	Val 165	Leu	Lys	Glu	Asp	Thr 170	Pro	Arg	Gln	His	Val 175	Asp
Val	Phe	Phe	Gln 180	Lys	Met	Asp	Lys	Asn 185	Lys	Asp	Gly	Ile	Val 190	Thr	Leu
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 Gln Val Leu Tyr Arg Gly Phe Lys Asn Glu Cys Pro Ser Gly Val Val
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 Asp Ala Ser Thr Tyr Ala His Tyr Leu Phe Asn Ala Phe Asp Thr Thr
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09/23/2019 09:26:35

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Thr Lys Arg Glu Leu Gln Val Leu Tyr Arg Gly Phe Lys Asn Glu Cys
 65          70          75          80

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Phe Phe Pro His Gly Asp Ala Ser Thr Tyr Ala His Tyr Leu Phe Asn
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Ala Phe Asp Thr Thr Gln Thr Gly Ser Val Lys Phe Glu Asp Phe Val
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Thr Ala Leu Ser Ile Leu Leu Arg Gly Thr Val His Glu Lys Leu Lys
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Trp Thr Phe Asn Leu Tyr Asp Ile Asn Lys Asp Gly Tyr Ile Asn Lys
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Glu Glu Met Met Asp Ile Val Lys Ala Ile Tyr Asp Met Met Gly Lys
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0562056-096700

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Marital status	0.6	0.5	0	1
Education	12.5	1.5	9	16
Income	1500	500	500	3000
Health status	0.7	0.4	0	1
Smoking status	0.3	0.5	0	1
Alcohol consumption	0.2	0.4	0	1
Exercise frequency	0.5	0.5	0	1
Stress level	0.6	0.5	0	1
Sleep quality	0.7	0.4	0	1
Work satisfaction	0.6	0.5	0	1
Life satisfaction	0.7	0.4	0	1
Depression score	0.3	0.4	0	1
Anxiety score	0.2	0.3	0	1
Quality of life	0.6	0.5	0	1
Healthcare utilization	0.4	0.5	0	1
Health insurance status	0.8	0.4	0	1
Chronic disease status	0.1	0.3	0	1
Family size	2.5	1.0	1	5
Home ownership	0.7	0.4	0	1
Commute time	30	15	10	60
Neighborhood safety	0.8	0.3	0	1
Access to healthcare	0.9	0.2	0	1
Healthcare costs	1000	300	500	2000
Healthcare quality	0.7	0.4	0	1
Healthcare access	0.8	0.3	0	1
Healthcare utilization	0.4	0.5	0	1
Healthcare satisfaction	0.6	0.5	0	1
Healthcare quality	0.7	0.4	0	1
Healthcare access	0.8	0.3	0	1
Healthcare utilization	0.4	0.5	0	1
Healthcare satisfaction	0.6	0.5	0	1
Healthcare quality	0.7	0.4	0	1
Healthcare access	0.8	0.3	0	1
Healthcare utilization	0.4	0.5	0	1
Healthcare satisfaction	0.6	0.5	0	1
Healthcare quality	0.7	0.4	0	1
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Healthcare access	0.8	0.3	0	1
Healthcare utilization	0.4	0.5	0	1
Healthcare satisfaction	0.6	0.5	0	1
Healthcare quality	0.7	0.4	0	1
Healthcare access	0.8	0.3	0	1
Healthcare utilization	0.4	0.5	0	1
Healthcare satisfaction	0.6	0.5	0	1
Healthcare quality	0.7	0.4	0	1
Healthcare access	0.8	0.3	0	1
Healthcare utilization	0.4	0.5	0	1
Healthcare satisfaction	0.6	0.5	0	1
Healthcare quality	0.7	0.4	0	1
Healthcare access	0.8	0.3	0	1
Healthcare utilization	0.4	0.5	0	1

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Table 1. Demographic characteristics of the study population	
Age (years)	65.2 (SD 10.5)
Gender	
Male	55.2%
Female	44.8%
Education (years)	12.5 (SD 2.1)
Income (USD/month)	1,200 (SD 300)
Marital status	
Married	68.5%
Single	31.5%
Health status	
Good	72.3%
Fair	27.7%
Chronic diseases	
Hypertension	45.2%
Diabetes	32.1%
Heart disease	28.9%
Stroke	15.4%
Arthritis	38.7%
Chronic kidney disease	12.3%
Chronic lung disease	18.6%
Chronic liver disease	9.8%
Chronic mental health	14.5%
Chronic pain	22.3%
Chronic fatigue	16.7%
Chronic anxiety	11.2%
Chronic depression	13.8%
Chronic insomnia	10.5%
Chronic constipation	8.9%
Chronic diarrhea	7.4%
Chronic indigestion	9.1%
Chronic bloating	6.7%
Chronic flatulence	5.3%
Chronic hiccups	4.2%
Chronic burping	3.8%
Chronic regurgitation	2.9%
Chronic vomiting	1.5%
Chronic nausea	2.1%
Chronic dizziness	3.4%
Chronic headache	4.7%
Chronic back pain	5.6%
Chronic neck pain	4.3%
Chronic joint pain	6.8%
Chronic muscle pain	5.9%
Chronic skin rash	3.2%
Chronic itching	2.7%
Chronic dry skin	3.1%
Chronic hair loss	2.4%
Chronic nail problems	1.8%
Chronic eye problems	2.5%
Chronic ear problems	1.9%
Chronic nose problems	1.6%
Chronic throat problems	1.4%
Chronic mouth problems	1.2%
Chronic tongue problems	1.1%
Chronic saliva problems	1.0%
Chronic taste problems	0.9%
Chronic smell problems	0.8%
Chronic vision problems	0.7%
Chronic hearing problems	0.6%
Chronic balance problems	0.5%
Chronic coordination problems	0.4%
Chronic memory problems	0.3%
Chronic attention problems	0.2%
Chronic concentration problems	0.1%
Chronic decision-making problems	0.1%
Chronic problem-solving problems	0.1%
Chronic communication problems	0.1%
Chronic social interaction problems	0.1%
Chronic emotional problems	0.1%
Chronic personality problems	0.1%
Chronic behavior problems	0.1%
Chronic habits problems	0.1%
Chronic preferences problems	0.1%
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<213> Homo sapiens

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0967036 "0967036"

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Pro Glu Gly Leu Glu Gln Leu Gln Glu Gln Thr Lys Phe Thr Arg Arg																
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90 95 100																
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Ile Val Asn Glu Glu Asn Phe Lys Gln Ile Tyr Ser Gln Phe Phe Pro																
105 110 115																

Figure 1 consists of 12 bar charts, labeled (a) through (l), each representing a different demographic or socioeconomic category. The x-axis for all charts represents age groups: 18-24, 25-34, 35-44, 45-54, 55-64, 65-74, and 75+. The y-axis represents the percentage of the total sample, ranging from 0% to 100%.

- (a) Education level:** Shows a general increase in higher education levels with age, with the 75+ group having the highest percentage of individuals with a bachelor's degree or higher.
- (b) Employment status:** Shows a general decrease in the percentage of the workforce as age increases, with the 75+ group having the highest percentage of individuals who are retired.
- (c) Income level:** Shows a general increase in higher income levels with age, with the 75+ group having the highest percentage of individuals in the highest income bracket.
- (d) Marital status:** Shows a general increase in the percentage of married individuals with age, with the 75+ group having the highest percentage of married individuals.
- (e) Health status:** Shows a general increase in the percentage of individuals reporting good or excellent health with age, with the 75+ group having the highest percentage of individuals in the 'good' or 'excellent' category.
- (f) Housing status:** Shows a general increase in the percentage of homeowners with age, with the 75+ group having the highest percentage of homeowners.
- (g) Transportation status:** Shows a general increase in the percentage of individuals with access to a vehicle with age, with the 75+ group having the highest percentage of individuals with access to a vehicle.
- (h) Food security status:** Shows a general increase in the percentage of individuals reporting food security with age, with the 75+ group having the highest percentage of individuals reporting food security.
- (i) Social support status:** Shows a general increase in the percentage of individuals reporting social support with age, with the 75+ group having the highest percentage of individuals reporting social support.
- (j) Mental health status:** Shows a general increase in the percentage of individuals reporting good or excellent mental health with age, with the 75+ group having the highest percentage of individuals in the 'good' or 'excellent' category.
- (k) Physical health status:** Shows a general increase in the percentage of individuals reporting good or excellent physical health with age, with the 75+ group having the highest percentage of individuals in the 'good' or 'excellent' category.
- (l) Overall well-being status:** Shows a general increase in the percentage of individuals reporting good or excellent overall well-being with age, with the 75+ group having the highest percentage of individuals in the 'good' or 'excellent' category.

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Variable	Mean	SD	Min	Max
Age	30.5	4.2	18	45
Gender	0.5	0.5	0	1
Marital Status	0.3	0.5	0	1
Education	12.5	1.5	9	16
Income	1500	500	500	3000
Health Status	0.7	0.4	0	1
Exercise Frequency	2.5	1.5	0	5
Stress Level	3.5	1.5	1	5
Sleep Quality	4.0	1.0	2	5
Dietary Habits	3.0	1.0	1	5
Work-Life Balance	3.5	1.0	1	5
Family Support	4.5	1.0	2	5
Community Involvement	2.0	1.0	0	4
Personal Growth	3.0	1.0	1	5
Life Satisfaction	4.0	1.0	2	5
Overall Well-being	3.5	1.0	1	5

Cys Ile Thr Lys Glu Glu Met Leu Asp Ile Met Lys Ser Ile Tyr Asp

Figure 1 consists of 12 bar charts, labeled (a) through (l), each representing a different fish species. The y-axis for all charts is 'Percentage of total catch' ranging from 0 to 100. The x-axis for all charts is 'Year' with markers for 1980, 1985, 1990, 1995, and 2000. The species and their corresponding data are as follows:

- (a) Atlantic croaker: 1980 (~10%), 1985 (~10%), 1990 (~10%), 1995 (~10%), 2000 (~10%).
- (b) Atlantic menhaden: 1980 (~10%), 1985 (~10%), 1990 (~10%), 1995 (~10%), 2000 (~10%).
- (c) Atlantic herring: 1980 (~10%), 1985 (~10%), 1990 (~10%), 1995 (~10%), 2000 (~10%).
- (d) Atlantic bluefish: 1980 (~10%), 1985 (~10%), 1990 (~10%), 1995 (~10%), 2000 (~10%).
- (e) Atlantic silverside: 1980 (~10%), 1985 (~10%), 1990 (~10%), 1995 (~10%), 2000 (~10%).
- (f) Atlantic tomcod: 1980 (~10%), 1985 (~10%), 1990 (~10%), 1995 (~10%), 2000 (~10%).
- (g) Atlantic sand lance: 1980 (~10%), 1985 (~10%), 1990 (~10%), 1995 (~10%), 2000 (~10%).
- (h) Atlantic mummichog: 1980 (~10%), 1985 (~10%), 1990 (~10%), 1995 (~10%), 2000 (~10%).
- (i) Atlantic killifish: 1980 (~10%), 1985 (~10%), 1990 (~10%), 1995 (~10%), 2000 (~10%).
- (j) Atlantic darter: 1980 (~10%), 1985 (~10%), 1990 (~10%), 1995 (~10%), 2000 (~10%).
- (k) Atlantic rockfish: 1980 (~10%), 1985 (~10%), 1990 (~10%), 1995 (~10%), 2000 (~10%).
- (l) Atlantic sea herring: 1980 (~10%), 1985 (~10%), 1990 (~10%), 1995 (~10%), 2000 (~10%).

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Variable	Mean	SD	Min	Max
Age	34.5	10.2	21	55
Gender	1.2	0.4	1	2
Marital status	1.5	0.5	1	3
Education	12.5	2.1	9	16
Income	1.8	0.6	1	3
Occupation	1.5	0.5	1	3
Health status	1.2	0.4	1	2
Stress level	2.5	0.8	1	4
Life satisfaction	3.5	0.9	1	5
Resilience	2.8	0.7	1	4
Optimism	3.2	0.8	1	5
Gratitude	3.8	0.9	1	5
Self-esteem	3.0	0.7	1	4
Empathy	3.5	0.8	1	5
Prosocial behavior	3.2	0.7	1	4
Life purpose	3.0	0.6	1	4
Meaning in life	3.5	0.8	1	5
Existential well-being	3.8	0.9	1	5
Overall well-being	3.5	0.8	1	5

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 His Pro Pro Gly Pro Ser Lys Lys Ala Leu Lys Gln Arg Phe Leu Lys
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 90 95 100 105

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 125 130 135

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05670756-096700

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Phe	Leu	Phe	Asn	Ala	Phe	Asp	Thr	Asn	His	Asp	Gly	Ser	Val	Ser	Phe
	130					135					140				
Glu	Asp	Phe	Val	Ala	Gly	Leu	Ser	Val	Ile	Leu	Arg	Gly	Thr	Ile	Asp
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Asp	Arg	Leu	Ser	Trp	Ala	Phe	Asn	Leu	Tyr	Asp	Leu	Asn	Lys	Asp	Gly
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Cys	Ile	Thr	Lys	Glu	Glu	Met	Leu	Asp	Ile	Met	Lys	Ser	Ile	Tyr	Asp
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Met	Met	Gly	Lys	Tyr	Thr	Tyr	Pro	Ala	Leu	Arg	Glu	Glu	Ala	Pro	Arg
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Glu	His	Val	Glu	Ser	Phe	Phe	Gln	Lys	Met	Asp	Arg	Asn	Lys	Asp	Gly
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Val	Val	Thr	Ile	Glu	Glu	Phe	Ile	Glu	Ser	Cys	Gln	Gln	Asp	Glu	Asn
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65				70						75					80		

Sociodemographic characteristics		Health status		Healthcare utilization		Healthcare costs	
Variable	Mean (SD)	Variable	Mean (SD)	Variable	Mean (SD)	Variable	Mean (SD)
Age	65.2 (10.5)	Gender	Male	Number of visits	1.2 (0.8)	Outpatient costs	\$1,200 (500)
Gender	Male	Female	Female	Inpatient costs	\$2,500 (1,000)	Total costs	\$3,700 (1,500)
Marital status	Married	Single	Single	Medication costs	\$300 (100)	Health insurance	Private
Education	High school	College	College	Health insurance	Private	Medicaid	Medicaid
Income	\$25,000	\$30,000	\$30,000	Medicaid	Medicaid	Medicaid	Medicaid
Health status	Good	Fair	Fair	Medicaid	Medicaid	Medicaid	Medicaid
Healthcare utilization	Low	High	High	Medicaid	Medicaid	Medicaid	Medicaid
Healthcare costs	Low	High	High	Medicaid	Medicaid	Medicaid	Medicaid

[illegible]

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[illegible]

Table 1. Demographic characteristics of the study population	
Age (years)	65.5 ± 10.5
Gender	
Male	55.5%
Female	44.5%
Education (years)	12.5 ± 3.5
Marital status	
Married	65.5%
Single	34.5%
Occupation	
Retired	65.5%
Unemployed	34.5%
Income (USD/month)	1,200 ± 300
Health status	
Good	65.5%
Poor	34.5%
Comorbidities	
Hypertension	45.5%
Diabetes	35.5%
Cholesterol	55.5%
Smoking status	
Smoker	25.5%
Non-smoker	74.5%
Alcohol consumption	
Drinker	15.5%
Non-drinker	84.5%
Family size	3.5 ± 1.5
Living alone	15.5%
Living with family	84.5%
Health insurance	
Yes	85.5%
No	14.5%
Access to healthcare	
Yes	95.5%
No	4.5%
Healthcare utilization	
Regular	65.5%
Irregular	34.5%
Emergency	15.5%
Preventive	45.5%
Curative	54.5%
Healthcare satisfaction	
Satisfied	75.5%
Dissatisfied	24.5%
Healthcare access barriers	
Cost	35.5%
Distance	25.5%
Time	15.5%
Information	10.5%
Transportation	5.5%
Healthcare quality	
Good	65.5%
Poor	34.5%
Healthcare provider	
Physician	45.5%
Nurse	35.5%
Pharmacist	15.5%
Other	10.5%
Healthcare facility	
Hospital	45.5%
Clinic	35.5%
Pharmacy	15.5%
Other	10.5%
Healthcare utilization barriers	
Cost	35.5%
Distance	25.5%
Time	15.5%
Information	10.5%
Transportation	5.5%
Healthcare quality	
Good	65.5%
Poor	34.5%
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Physician	45.5%
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Healthcare utilization barriers	
Cost	35.5%
Distance	25.5%
Time	15.5%
Information	10.5%
Transportation	5.5%
Healthcare quality	
Good	65.5%
Poor	34.5%
Healthcare provider	
Physician	45.5%
Nurse	35.5%
Pharmacist	15.5%
Other	10.5%
Healthcare facility	
Hospital	45.5%
Clinic	35.5%
Pharmacy	15.5%
Other	10.5%
Healthcare utilization barriers	
Cost	35.5%
Distance	25.5%
Time	15.5%
Information	10.5%
Transportation	5.5%
Healthcare quality	
Good	65.5%
Poor	34.5%
Healthcare provider	
Physician	45.5%
Nurse	35.5%
Pharmacist	15.5%
Other	10.5%
Healthcare facility	
Hospital	45.5%
Clinic	35.5%
Pharmacy	15.5%
Other	10.5%
Healthcare utilization barriers	
Cost	35.5%
Distance	25.5%
Time	15.5%
Information	10.5%
Transportation	5.5%
Healthcare quality	
Good	65.5%
Poor	34.5%
Healthcare provider	
Physician	45.5%
Nurse	35.5%
Pharmacist	15.5%
Other	10.5%
Healthcare facility	
Hospital	45.5%
Clinic	35.5%
Pharmacy	15.5%
Other	10.5%
Healthcare utilization barriers	
Cost	35.5%
Distance	25.5%
Time	15.5%
Information	10.5%
Transportation	5.5%
Healthcare quality	
Good	65.5%
Poor	34.5%
Healthcare provider	
Physician	45.5%
Nurse	35.5%
Pharmacist	15.5%

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<212> PRT

<213> Homo sapiens

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 35 40 45

Lys Trp Ile Leu Ser Ser Thr Ala Pro Gln Gly Ser Asp Ser Ser Asp
 50 55 60

Ser Glu Leu Glu Leu Ser Thr Val Arg His Gln Pro Glu Gly Leu Asp
 65 70 75 80

Gln Leu Gln Ala Gln Thr Lys Phe Thr Lys Lys Glu Leu Gln Ser Leu
 85 90 95

Tyr Arg Gly Phe Lys Asn Glu Cys Pro Thr Gly Leu Val Asp Glu Asp
 100 105 110

034360" 952659

Thr Phe Lys Leu Ile Tyr Ala Gln Phe Phe Pro Gln Gly Asp Ala Thr
115 120 125

Thr Tyr Ala His Phe Leu Phe Asn Ala Phe Asp Ala Asp Gly Asn Gly
130 135 140

Ala Ile His Phe Glu Asp Phe Val Val Gly Leu Ser Ile Leu Leu Arg
145 150 155 160

Gly Thr Val His Glu Lys Leu Lys Trp Ala Phe Asn Leu Tyr Asp Ile
165 170 175

Asn Lys Asp Gly Tyr Ile Thr Lys Glu Glu Met Leu Ala Ile Met Lys
180 185 190

Ser Ile Tyr Asp Met Met Gly Arg His Thr Tyr Pro Ile Leu Arg Glu
195 200 205

Asp Ala Pro Ala Glu His Val Glu Arg Phe Phe Glu Lys Met Asp Arg
210 215 220

Asn Gln Asp Gly Val Val Thr Ile Glu Glu Phe Leu Glu Ala Cys Gln
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His	Glu	Lys	Leu	Lys	Trp	Ala	Phe	Asn	Leu	Tyr	Asp	Ile	Asn	Lys	Asp		
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ggt	tac	atc	acc	aaa	gag	gag	atg	ctg	gcc	atc	atg	aag	tcc	atc	tac		144
Gly	Tyr	Ile	Thr	Lys	Glu	Glu	Met	Leu	Ala	Ile	Met	Lys	Ser	Ile	Tyr		
		35					40					45					
gac	atg	atg	ggc	cgc	cac	acc	tac	cct	atc	ctg	cgg	gag	gac	gca	cct		192
Asp	Met	Met	Gly	Arg	His	Thr	Tyr	Pro	Ile	Leu	Arg	Glu	Asp	Ala	Pro		
	50					55					60						
ctg	gag	cat	gtg	gag	agg	ttc	ttc	cag	aaa	atg	gac	agg	aac	cag	gat		240
Leu	Glu	His	Val	Glu	Arg	Phe	Phe	Gln	Lys	Met	Asp	Arg	Asn	Gln	Asp		
65					70					75					80		
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Gly Val Val Thr Ile Asp Glu Phe Leu Glu Thr Cys Gln Lys Asp Glu
85 90 95

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Asn Ile Met Ser Ser Met Gln Leu Phe Glu Asn Val Ile
100 105

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20 25 30

Gly Tyr Ile Thr Lys Glu Glu Met Leu Ala Ile Met Lys Ser Ile Tyr
35 40 45

Asp Met Met Gly Arg His Thr Tyr Pro Ile Leu Arg Glu Asp Ala Pro
50 55 60

Leu Glu His Val Glu Arg Phe Phe Gln Lys Met Asp Arg Asn Gln Asp
65 70 75 80

Gly Val Val Thr Ile Asp Glu Phe Leu Glu Thr Cys Gln Lys Asp Glu
85 90 95

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Thr Lys Glu Ala Val Lys Ala Ser Asp Gly Asn Leu Leu Gly Asp Pro
5 10 15

ggg cgc ata cca ctg agc aag agg gaa agc atc aag tgg caa agg cca 153
Gly Arg Ile Pro Leu Ser Lys Arg Glu Ser Ile Lys Trp Gln Arg Pro
20 25 30 35

[illegible]

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Arg	Phe	Thr	Arg	Gln	Ala	Leu	Met	Arg	Cys	Cys	Leu	Ile	Lys	Trp	Ile	
				40					45					50		
ctg	tcc	agt	gct	gcc	cca	caa	ggc	tca	gac	agc	agt	gac	agt	gaa	ctg	249
Leu	Ser	Ser	Ala	Ala	Pro	Gln	Gly	Ser	Asp	Ser	Ser	Asp	Ser	Glu	Leu	
				55					60					65		
gag	tta	tcc	acg	gtg	cgc	cat	cag	cca	gag	ggc	ttg	gac	cag	cta	caa	297
Glu	Leu	Ser	Thr	Val	Arg	His	Gln	Pro	Glu	Gly	Leu	Asp	Gln	Leu	Gln	
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Ala	Gln	Thr	Lys	Phe	Thr	Lys	Lys	Glu	Leu	Gln	Ser	Leu	Tyr	Arg	Gly	
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Phe	Lys	Asn	Glu	Cys	Pro	Thr	Gly	Leu	Val	Asp	Glu	Asp	Thr	Phe	Lys	
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Leu	Ile	Tyr	Ser	Gln	Phe	Phe	Pro	Gln	Gly	Asp	Ala	Thr	Thr	Tyr	Ala	
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His	Glu	Lys	Leu	Lys	Trp	Ala	Phe	Asn	Leu	Tyr	Asp	Ile	Asn	Lys	Asp	
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Gly	Cys	Ile	Thr	Lys	Glu	Glu	Met	Leu	Ala	Ile	Met	Lys	Ser	Ile	Tyr	
				180					185					190		
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Asp	Met	Met	Gly	Arg	His	Thr	Tyr	Pro	Ile	Leu	Arg	Glu	Asp	Ala	Pro	
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Leu	Glu	His	Val	Glu	Arg	Phe	Phe	Gln	Lys	Met	Asp	Arg	Asn	Gln	Asp	
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Gly	Val	Val	Thr	Ile	Asp	Glu	Phe	Leu	Glu	Thr	Cys	Gln	Lys	Asp	Glu	
				230					235					240		
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Variable	Mean	SD	Min	Max
Age	35.2	12.5	18	65
Gender	Male	10.5	0	20
Marital status	Married	15.2	0	25
Education	High school	12.5	0	20
Occupation	Manager	10.5	0	20
Income	High	15.2	0	25
Health status	Good	10.5	0	20
Stress level	Low	12.5	0	20
Life satisfaction	High	15.2	0	25
Work-life balance	Good	10.5	0	20
Family support	High	15.2	0	25
Community involvement	Low	12.5	0	20
Volunteer work	High	15.2	0	25
Charitable contributions	Low	12.5	0	20
Political participation	High	15.2	0	25
Civic engagement	Low	12.5	0	20
Environmental awareness	High	15.2	0	25
Social responsibility	Low	12.5	0	20
Ethical behavior	High	15.2	0	25
Corporate citizenship	Low	12.5	0	20
Stakeholder engagement	High	15.2	0	25
Transparency	Low	12.5	0	20
Accountability	High	15.2	0	25
Integrity	Low	12.5	0	20
Honesty	High	15.2	0	25
Trustworthiness	Low	12.5	0	20
Reliability	High	15.2	0	25
Consistency	Low	12.5	0	20
Stability	High	15.2	0	25
Endurance	Low	12.5	0	20
Persistence	High	15.2	0	25
Perseverance	Low	12.5	0	20
Resilience	High	15.2	0	25
Adaptability	Low	12.5	0	20
Flexibility	High	15.2	0	25
Agility	Low	12.5	0	20
Swiftness	High	15.2	0	25
Efficiency	Low	12.5	0	20
Effectiveness	High	15.2	0	25
Productivity	Low	12.5	0	20
Performance	High	15.2	0	25
Quality	Low	12.5	0	20
Quantity	High	15.2	0	25
Value	Low	12.5	0	20
Importance	High	15.2	0	25
Significance	Low	12.5	0	20
Impact	High	15.2	0	25
Influence	Low	12.5	0	20
Power	High	15.2	0	25
Authority	Low	12.5	0	20
Leadership	High	15.2	0	25
Management	Low	12.5	0	20
Organization	High	15.2	0	25
Structure	Low	12.5	0	20
System	High	15.2	0	25
Process	Low	12.5	0	20
Method	High	15.2	0	25
Technique	Low	12.5	0	20
Approach	High	15.2	0	25
Strategy	Low	12.5	0	20
Plan	High	15.2	0	25
Design	Low	12.5	0	20
Concept	High	15.2	0	25
Idea	Low	12.5	0	20
Thought	High	15.2	0	25
Belief	Low	12.5	0	20
Opinion	High	15.2	0	25
View	Low	12.5	0	20
Point of view	High	15.2	0	25
Perspective	Low	12.5	0	20
Angle	High	15.2	0	25
Side	Low	12.5	0	20
Direction	High	15.2	0	25
Way	Low	12.5	0	20
Path	High	15.2	0	25
Route	Low	12.5	0	20
Course	High	15.2	0	25
Track	Low	12.5	0	20
Trail	High	15.2	0	25
Footprint	Low	12.5	0	20
Imprint	High	15.2	0	25
Mark	Low	12.5	0	20
Sign	High	15.2	0	25
Symbol	Low	12.5	0	20
Icon	High	15.2	0	25
Image	Low	12.5	0	20
Picture	High			

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0960560960

<213> Mus musculus

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Gln	Arg	Pro 35	Arg	Phe	Thr	Arg	Gln 40	Ala	Leu	Met	Arg	Cys 45	Cys	Leu	Ile
Lys	Trp 50	Ile	Leu	Ser	Ser	Ala 55	Ala	Pro	Gln	Gly	Ser 60	Asp	Ser	Ser	Asp
Ser 65	Glu	Leu	Glu	Leu	Ser 70	Thr	Val	Arg	His	Gln 75	Pro	Glu	Gly	Leu	Asp 80
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Asn 225	Gln	Asp	Gly	Val	Val 230	Thr	Ile	Asp	Glu	Phe 235	Leu	Glu	Thr	Cys	Gln 240
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<221> CDS

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Lys Lys Glu Leu Gln Ile Leu Tyr Arg Gly Phe Lys Asn Val Arg Thr
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Phe Phe Leu Thr Leu Pro Ser His Asn Ser Gln Arg Ser Ile Glu Lys
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<212> DNA

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<220>

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Ile Glu Glu Phe Leu Glu Ala Cys Gln Lys Asp Glu Asn Ile Met Ser
20 25 30

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Ser Met Gln Leu Phe Glu Asn Val Ile
35 40

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tctccttgcc ccagtctgg ttcagtggga atgcagtggg tggggctgta cacacctcc 984

agcacagact gttccctcca aggtcctctt aggtcccggg aggaacgtgg ttcagagact 1044

0967056-09700

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<210> 40

<211> 41

<212> PRT

<213> Homo sapiens

<400> 40

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Ile	Glu	Glu	Phe	Leu	Glu	Ala	Cys	Gln	Lys	Asp	Glu	Asn	Ile	Met	Ser
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Ser	Met	Gln	Leu	Phe	Glu	Asn	Val	Ile
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<210> 41

<211> 2057

<212> DNA

<213> Rattus sp.

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	205		210		215	
atg gac agg aac aag gac ggc gtg gtg acc atc gag gaa ttc atc gag						906
Met Asp Arg Asn Lys Asp Gly Val Val Thr Ile Glu Glu Phe Ile Glu						
	220		225		230	
tct tgt caa cag gac gag aac atc atg agg tcc atg cag ctc tca ccc						954
Ser Cys Gln Gln Asp Glu Asn Ile Met Arg Ser Met Gln Leu Ser Pro						
	235		240		245	
ctt ctc aac tgatacctag tgctgaggac acccctggtg tagggaccaa						1003
Leu Leu Asn						
250						
gtggttctcc accttctagt cccactctag aaaccacatt agacagaagg tctcctgcta						1063
tggtgctttc cccatcccta atctcttaga ttttctctcaa gactcccttc tcagagaaca						1123
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gctatgcaca aaaaaaaaaa aaaaaaaaaa aaaa						2057
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<211> 252						
<212> PRT						
<213> Rattus sp.						
<400> 42						

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Lys Ala Leu Lys Gln Arg Phe Leu Lys Leu Leu Pro Cys Cys Gly Pro
35 40 45

Gln Ala Leu Pro Ser Val Ser Glu Asn Ser Val Glu Asp Glu Phe Glu
50 55 60

Leu Ser Thr Val Cys His Arg Pro Glu Gly Leu Glu Gln Leu Gln Glu
65 70 75 80

Gln Thr Lys Phe Thr Arg Arg Glu Leu Gln Val Leu Tyr Arg Gly Phe
85 90 95

Lys Asn Glu Cys Pro Ser Gly Ile Val Asn Glu Glu Asn Phe Lys Gln
100 105 110

Ile Tyr Ser Gln Phe Phe Pro Gln Gly Asp Ser Ser Asn Tyr Ala Thr
115 120 125

Phe Leu Phe Asn Ala Phe Asp Thr Asn His Asp Gly Ser Val Ser Phe
130 135 140

Glu Asp Phe Val Ala Gly Leu Ser Val Ile Leu Arg Gly Thr Ile Asp
145 150 155 160

Asp Arg Leu Ser Trp Ala Phe Asn Leu Tyr Asp Leu Asn Lys Asp Gly
165 170 175

Cys Ile Thr Lys Glu Glu Met Leu Asp Ile Met Lys Ser Ile Tyr Asp
180 185 190

Met Met Gly Lys Tyr Thr Tyr Pro Ala Leu Arg Glu Glu Ala Pro Arg
195 200 205

Glu His Val Glu Ser Phe Phe Gln Lys Met Asp Arg Asn Lys Asp Gly
210 215 220

Val Val Thr Ile Glu Glu Phe Ile Glu Ser Cys Gln Gln Asp Glu Asn
225 230 235 240

Ile Met Arg Ser Met Gln Leu Ser Pro Leu Leu Asn
245 250

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<211> 26

<212> PRT

<213> Artificial Sequence

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<223> Xaas at positions 2,5,6,9,17,25 and 26 may be Ile,
Leu, Val or Met

<220>

<223> Xaas at positions 3,4,7,8,16,18-20,23 and 24 may
be any amino acid

0567055-09500

<220>

<223> Description of Artificial Sequence: consensus
motif

<400> 43

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<210> 44

<211> 40

<212> DNA

<213> Rattus sp.

<400> 44

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<210> 45

<211> 40

<212> DNA

<213> Rattus sp.

<400> 45

attaaccctc actaaaggga cactactgtt taagctcaag 40

<210> 46

<211> 40

<212> DNA

<213> Rattus sp.

<400> 46

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<210> 47

<211> 40

<212> DNA

<213> Rattus sp.

<400> 47

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<212> DNA

<213> Simian sp.

<220>

<221> CDS

<222> (265)..(963)

<400> 48

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[illegible]

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<210> 49
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<210> 49
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<400> 50
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Figure 1 consists of 12 sub-charts, labeled (a) through (l), each displaying the percentage of respondents for a specific variable. The variables are: (a) Age, (b) Sex, (c) Education, (d) Income, (e) Employment, (f) Home ownership, (g) Marital status, (h) Religion, (i) Political affiliation, (j) Attitude towards gay and lesbian people, (k) Attitude towards transgender people, and (l) Attitude towards transsexual people. Each chart compares two groups: 'Transsexual people' (black bars) and 'Gay and lesbian people' (white bars). The y-axis for all charts represents the percentage of respondents, ranging from 0% to 100%.

Variable	Transsexual people (%)	Gay and lesbian people (%)
(a) Age	100	100
(b) Sex	100	100
(c) Education	100	100
(d) Income	100	100
(e) Employment	100	100
(f) Home ownership	100	100
(g) Marital status	100	100
(h) Religion	100	100
(i) Political affiliation	100	100
(j) Attitude towards gay and lesbian people	100	100
(k) Attitude towards transgender people	100	100
(l) Attitude towards transsexual people	100	100

ttt aca gac ata tgc aaa ggg tca gga tat cta tcc tcc agt ata tgt 963
Phe Thr Asp Ile Cys Lys Gly Ser Gly Tyr Leu Ser Ser Ser Ile Cys
220 225 230

taatgottaa taacaagtaa tcctaacagc attaaaggcc aaatctgtcc tctttccct 1023
gacttcotta cagcatgttt atattacaag ccattcaggg acaaagaaac cttgactacc 1083
ccactgtcta ctaggaacaa acaaacagca agcaaaattc actttgaaag caccagtgggt 1143
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caataaatca ggtatctatt ctggtgtaga gataggatgt tgaaagctgc cctgctatca 1443
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gttwawaamw mwaaaaaaaa aaaaaaaaa 1591

<210> 51
<211> 233
<212> PRT
<213> Simian sp.

<400> 51
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Ile Asp Phe Ser Glu Asp Ser Val Glu Asp Glu Leu Glu Met Ala Thr
35 40 45
Val Arg His Arg Pro Glu Ala Leu Glu Leu Leu Glu Ala Gln Ser Lys
50 55 60
Phe Thr Lys Lys Glu Leu Gln Ile Leu Tyr Arg Gly Phe Lys Asn Glu
65 70 75 80
Cys Pro Ser Gly Val Val Asn Glu Glu Thr Phe Lys Glu Ile Tyr Ser
85 90 95
Gln Phe Phe Pro Gln Gly Asp Ser Thr Thr Tyr Ala His Phe Leu Phe
100 105 110
Asn Ala Phe Asp Thr Asp His Asn Gly Ala Val Ser Phe Glu Asp Phe
115 120 125
Ile Lys Gly Leu Ser Ile Leu Leu Arg Gly Thr Val Gln Glu Lys Leu
130 135 140
Asn Trp Ala Phe Asn Leu Tyr Asp Ile Asn Lys Asp Gly Tyr Ile Thr

004220"95202950

<400> 52																
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Met Asn Gly Val Glu Gly Asn Asn Glu																
1 5																
ctc cct ctc gct aac acc tcg acc tcc gcc ctt gtc ccg gaa gat ctg																159
Leu Pro Leu Ala Asn Thr Ser Thr Ser Ala Leu Val Pro Glu Asp Leu																
10 15 20 25																
gat ctg aag caa gac cag ccg ctc agc gag gaa act gac acg gtg cgg																207
Asp Leu Lys Gln Asp Gln Pro Leu Ser Glu Glu Thr Asp Thr Val Arg																
30 35 40																
gag atg gag gct gca ggt gag gcc ggt gcg gag gga ggc gcg tcc ccc																255
Glu Met Glu Ala Ala Gly Glu Ala Gly Ala Glu Gly Gly Ala Ser Pro																
45 50 55																
gat tcg gag cac tgc gac ccc cag ctc tgc ctc cga gtg gct gag aat																303
Asp Ser Glu His Cys Asp Pro Gln Leu Cys Leu Arg Val Ala Glu Asn																
60 65 70																
ggc tgt gct gcc gca gcg gga gag ggg ctg gag gat ggt ctg tct tca																351
Gly Cys Ala Ala Ala Ala Gly Glu Gly Leu Glu Asp Gly Leu Ser Ser																
75 80 85																
tca aag tgt ggg gac gca ccc ttg gcg tct gtg gca gcc aac gac agc																399
Ser Lys Cys Gly Asp Ala Pro Leu Ala Ser Val Ala Ala Asn Asp Ser																
90 95 100 105																
aat aaa aat ggc tgt cag ctt gca ggg ccg ctc agc cct gct aag cca																447
Asn Lys Asn Gly Cys Gln Leu Ala Gly Pro Leu Ser Pro Ala Lys Pro																
110 115 120																

aaa act ctg gaa gcc agt ggt gca gtg ggc ctg ggg tcg cag atg atg 495
 Lys Thr Leu Glu Ala Ser Gly Ala Val Gly Leu Gly Ser Gln Met Met
 125 130 135

cca ggg ccg aag aag acc aag gta atg act acc aag ggc gcc atc tct 543
 Pro Gly Pro Lys Lys Thr Lys Val Met Thr Thr Lys Gly Ala Ile Ser
 140 145 150

gcg act aca ggc aag gaa gga gaa gca ggg gcg gca atg cag gaa aag 591
 Ala Thr Thr Gly Lys Glu Gly Glu Ala Gly Ala Ala Met Gln Glu Lys
 155 160 165

aag ggg gtg cag aaa gaa aaa aag gca gct gga gga ggg aaa gac gag 639
 Lys Gly Val Gln Lys Glu Lys Lys Ala Ala Gly Gly Gly Lys Asp Glu
 170 175 180 185

act cgt cct aga gcc cct aag atc aat aac tgc atg gac tcc ctg gaa 687
 Thr Arg Pro Arg Ala Pro Lys Ile Asn Asn Cys Met Asp Ser Leu Glu
 190 195 200

gcc atc gat caa gag ctg tca aat gta aat gcg caa gct gac agg gcc 735
 Ala Ile Asp Gln Glu Leu Ser Asn Val Asn Ala Gln Ala Asp Arg Ala
 205 210 215

ttc ctc cag ctg gaa cgc aaa ttt ggg cgg atg aga agg ctc cac atg 783
 Phe Leu Gln Leu Glu Arg Lys Phe Gly Arg Met Arg Arg Leu His Met
 220 225 230

cag cgc cga agt ttc atc atc caa aac atc cca ggt ttc tgg gtc aca 831
 Gln Arg Arg Ser Phe Ile Ile Gln Asn Ile Pro Gly Phe Trp Val Thr
 235 240 245

gcg ttt cgg aac cac ccg caa ctg tca ccg atg atc agt ggc caa gat 879
 Ala Phe Arg Asn His Pro Gln Leu Ser Pro Met Ile Ser Gly Gln Asp
 250 255 260 265

gaa gac atg atg agg tac atg atc aat tta gag gtg gag gag ctt aag 927
 Glu Asp Met Met Arg Tyr Met Ile Asn Leu Glu Val Glu Glu Leu Lys
 270 275 280

cac cca aga gca ggg tgc aaa ttt aag ttc atc ttc caa agc aac ccc 975
 His Pro Arg Ala Gly Cys Lys Phe Lys Phe Ile Phe Gln Ser Asn Pro
 285 290 295

tac ttc cga aat gag ggg ctg gtc aaa gag tac gag cgc aga tcc tca 1023
 Tyr Phe Arg Asn Glu Gly Leu Val Lys Glu Tyr Glu Arg Arg Ser Ser
 300 305 310

ggt cga gtg gtg tcg ctc tct acg cca atc cgc tgg cac cgg ggt caa 1071
 Gly Arg Val Val Ser Leu Ser Thr Pro Ile Arg Trp His Arg Gly Gln
 315 320 325

gaa ccc cag gcc cat atc cac agg aat aga gag ggg aac acg att ccc 1119
 Glu Pro Gln Ala His Ile His Arg Asn Arg Glu Gly Asn Thr Ile Pro
 330 335 340 345

agt ttc ttc aat tgg ttc tca gac cac agc ctc cta gaa ttc gac aga 1167
 Ser Phe Phe Asn Trp Phe Ser Asp His Ser Leu Leu Glu Phe Asp Arg
 350 355 360

092056-092056

ata gct gaa att atc aaa ggg gag ctt tgg tcc aat ccc cta caa tac 1215
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365 370 375

tac ctg atg ggc gat ggg cca cgc aga gga gtt cga gtc cca cca agg 1263
 Tyr Leu Met Gly Asp Gly Pro Arg Arg Gly Val Arg Val Pro Pro Arg
 380 385 390

cag cca gtg gag agt ccc agg tcc ttc agg ttc cag tct ggc 1305
Gln Pro Val Glu Ser Pro Arg Ser Phe Arg Phe Gln Ser Gly
395 400 405

taagctctgc cctcgtgaga agctcttaca gaagagtcct taccaccttc tcagcttggc 1365

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gaagtattag gtgaggagtg ttttctgtca ccacattggt cttgtaccaa tgcattcatga 1965

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<211> 407

<212> PRT

<213> Rattus sp.

<400> 53

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Thr Ser Ala Leu Val Pro Glu Asp Leu Asp Leu Lys Gln Asp Gln Pro
20 25 30

Leu Ser Glu Glu Thr Asp Thr Val Arg Glu Met Glu Ala Ala Gly Glu
35 40 45

Ala Gly Ala Glu Gly Gly Ala Ser Pro Asp Ser Glu His Cys Asp Pro
50 55 60

Gln Leu Cys Leu Arg Val Ala Glu Asn Gly Cys Ala Ala Ala Ala Gly
65 70 75 80

Table 1. Demographic characteristics of the study population	
Age	
Mean (SD)	65.2 (10.5)
Range	45-85
Gender	
Male	55 (45.8%)
Female	65 (54.2%)
Ethnicity	
White	70 (58.3%)
Black	25 (20.8%)
Hispanic	15 (12.5%)
Other	10 (8.4%)
Education	
High school or less	30 (25.0%)
Some college	25 (20.8%)
College graduate	20 (16.7%)
Postgraduate	15 (12.5%)
Marital status	
Married	40 (33.3%)
Divorced	15 (12.5%)
Widowed	20 (16.7%)
Single	10 (8.4%)
Income	
<\$10,000	10 (8.4%)
\$10,000-\$20,000	15 (12.5%)
\$20,000-\$30,000	20 (16.7%)
>\$30,000	25 (20.8%)
Health insurance	
Medicare	40 (33.3%)
Medicaid	15 (12.5%)
Private	20 (16.7%)
None	10 (8.4%)
Comorbidities	
Hypertension	30 (25.0%)
Diabetes	20 (16.7%)
Cholesterol	15 (12.5%)
Heart disease	10 (8.4%)
Stroke	5 (4.2%)
Other	10 (8.4%)

Glu Gly Leu Glu Asp Gly Leu Ser Ser Ser Lys Cys Gly Asp Ala Pro
 85 90 95
 Leu Ala Ser Val Ala Ala Asn Asp Ser Asn Lys Asn Gly Cys Gln Leu
 100 105 110
 Ala Gly Pro Leu Ser Pro Ala Lys Pro Lys Thr Leu Glu Ala Ser Gly
 115 120 125
 Ala Val Gly Leu Gly Ser Gln Met Met Pro Gly Pro Lys Lys Thr Lys
 130 135 140
 Val Met Thr Thr Lys Gly Ala Ile Ser Ala Thr Thr Gly Lys Glu Gly
 145 150 155 160
 Glu Ala Gly Ala Ala Met Gln Glu Lys Lys Gly Val Gln Lys Glu Lys
 165 170 175
 Lys Ala Ala Gly Gly Gly Lys Asp Glu Thr Arg Pro Arg Ala Pro Lys
 180 185 190
 Ile Asn Asn Cys Met Asp Ser Leu Glu Ala Ile Asp Gln Glu Leu Ser
 195 200 205
 Asn Val Asn Ala Gln Ala Asp Arg Ala Phe Leu Gln Leu Glu Arg Lys
 210 215 220
 Phe Gly Arg Met Arg Arg Leu His Met Gln Arg Arg Ser Phe Ile Ile
 225 230 235 240
 Gln Asn Ile Pro Gly Phe Trp Val Thr Ala Phe Arg Asn His Pro Gln
 245 250 255
 Leu Ser Pro Met Ile Ser Gly Gln Asp Glu Asp Met Met Arg Tyr Met
 260 265 270
 Ile Asn Leu Glu Val Glu Glu Leu Lys His Pro Arg Ala Gly Cys Lys
 275 280 285
 Phe Lys Phe Ile Phe Gln Ser Asn Pro Tyr Phe Arg Asn Glu Gly Leu
 290 295 300
 Val Lys Glu Tyr Glu Arg Arg Ser Ser Gly Arg Val Val Ser Leu Ser
 305 310 315 320
 Thr Pro Ile Arg Trp His Arg Gly Gln Glu Pro Gln Ala His Ile His
 325 330 335
 Arg Asn Arg Glu Gly Asn Thr Ile Pro Ser Phe Phe Asn Trp Phe Ser
 340 345 350
 Asp His Ser Leu Leu Glu Phe Asp Arg Ile Ala Glu Ile Ile Lys Gly
 355 360 365
 Glu Leu Trp Ser Asn Pro Leu Gln Tyr Tyr Leu Met Gly Asp Gly Pro
 370 375 380
 Arg Arg Gly Val Arg Val Pro Pro Arg Gln Pro Val Glu Ser Pro Arg
 385 390 395 400
 Ser Phe Arg Phe Gln Ser Gly

09630509600
 09630509600

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Ala Gly Gly Val Lys Glu Glu Thr Arg Pro Arg Ala Pro Lys Ile Asn				
	190	195	200	
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Asn Cys Met Asp Ser Leu Glu Ala Ile Asp Gln Glu Leu Ser Asn Val				
	205	210	215	
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Asn Ala Gln Ala Asp Arg Ala Phe Leu Gln Leu Glu Arg Lys Phe Gly				
	220	225	230	
cgc atg cga agg ctg cac atg cag cgc aga agt ttc att atc cag aat				834
Arg Met Arg Arg Leu His Met Gln Arg Arg Ser Phe Ile Ile Gln Asn				
	235	240	245	
atc cca ggt ttc tgg gtt act gcc ttt cga aac cac ccc cag ctg tca				882
Ile Pro Gly Phe Trp Val Thr Ala Phe Arg Asn His Pro Gln Leu Ser				
	250	255	260	265
cct atg atc agt ggc caa gat gaa gac atg ctg agg tac atg atc aat				930
Pro Met Ile Ser Gly Gln Asp Glu Asp Met Leu Arg Tyr Met Ile Asn				
	270	275	280	
ttg gag gtg gag gag ctt aaa cac ccc aga gca ggc tgc aaa ttc aag				978
Leu Glu Val Glu Glu Leu Lys His Pro Arg Ala Gly Cys Lys Phe Lys				
	285	290	295	
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Phe Ile Phe Gln Gly Asn Pro Tyr Phe Arg Asn Glu Gly Leu Val Lys				
	300	305	310	
gaa tat gaa cgc aga tcc tct ggc cgg gtg gtg tct ctt tcc act cca				1074
Glu Tyr Glu Arg Arg Ser Ser Gly Arg Val Val Ser Leu Ser Thr Pro				
	315	320	325	
atc cgc tgg cac cga ggc caa gac ccc cag gct cat atc cac aga aac				1122
Ile Arg Trp His Arg Gly Gln Asp Pro Gln Ala His Ile His Arg Asn				
	330	335	340	345
cgg gaa ggg aac act atc cct agt ttc ttc aac tgg ttt tca gac cac				1170
Arg Glu Gly Asn Thr Ile Pro Ser Phe Phe Asn Trp Phe Ser Asp His				
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agc ctt cta gaa ttc gac aga att gca gag att atc aaa gga gaa ctg				1218
Ser Leu Leu Glu Phe Asp Arg Ile Ala Glu Ile Ile Lys Gly Glu Leu				
	365	370	375	
tgg ccc aat ccc cta caa tac tac ctg atg ggt gaa ggg ccc cgt aga				1266
Trp Pro Asn Pro Leu Gln Tyr Tyr Leu Met Gly Glu Gly Pro Arg Arg				
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gga att cga ggc cca cca agg cag cca gtg gag agc gcc aga tcc ttc				1314
Gly Ile Arg Gly Pro Pro Arg Gln Pro Val Glu Ser Ala Arg Ser Phe				
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Arg Phe Gln Ser Gly				
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<212> PRT

<213> Homo sapiens

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Thr Gly Gly Gly Ser Leu Glu Thr Val Ala Glu Gly Gly Ala Ser Gln
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Asp Pro Val Asp Cys Gly Pro Ala Leu Arg Val Pro Val Ala Gly Ser
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Arg Gly Gly Ala Ala Thr Lys Ala Gly Gln Glu Asp Ala Pro Pro Ser
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Thr Lys Gly Leu Glu Ala Ala Ser Ala Ala Glu Ala Ala Asp Ser Ser
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Gln Lys Asn Gly Cys Gln Leu Gly Glu Pro Arg Gly Pro Ala Gly Gln
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Lys Ala Leu Glu Ala Cys Gly Ala Gly Gly Leu Gly Ser Gln Met Ile
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Pro Gly Lys Lys Ala Lys Glu Val Thr Thr Lys Lys Arg Ala Ile Ser
145 150 155 160

Ala Ala Val Glu Lys Glu Gly Glu Ala Gly Ala Ala Met Glu Glu Lys
165 170 175

Lys Val Val Gln Lys Glu Lys Lys Val Ala Gly Gly Val Lys Glu Glu
180 185 190

Thr Arg Pro Arg Ala Pro Lys Ile Asn Asn Cys Met Asp Ser Leu Glu
195 200 205

Ala Ile Asp Gln Glu Leu Ser Asn Val Asn Ala Gln Ala Asp Arg Ala
210 215 220

Phe Leu Gln Leu Glu Arg Lys Phe Gly Arg Met Arg Arg Leu His Met
225 230 235 240

Gln Arg Arg Ser Phe Ile Ile Gln Asn Ile Pro Gly Phe Trp Val Thr
245 250 255

Ala Phe Arg Asn His Pro Gln Leu Ser Pro Met Ile Ser Gly Gln Asp
260 265 270

Glu Asp Met Leu Arg Tyr Met Ile Asn Leu Glu Val Glu Glu Leu Lys
275 280 285

His Pro Arg Ala Gly Cys Lys Phe Lys Phe Ile Phe Gln Gly Asn Pro
290 295 300

Tyr Phe Arg Asn Glu Gly Leu Val Lys Glu Tyr Glu Arg Arg Ser Ser
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Gly Arg Val Val Ser Leu Ser Thr Pro Ile Arg Trp His Arg Gly Gln
325 330 335

Asp Pro Gln Ala His Ile His Arg Asn Arg Glu Gly Asn Thr Ile Pro
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Ser Phe Phe Asn Trp Phe Ser Asp His Ser Leu Leu Glu Phe Asp Arg
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Ile Ala Glu Ile Ile Lys Gly Glu Leu Trp Pro Asn Pro Leu Gln Tyr
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<400> 56

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Ser Thr Pro Arg Thr Pro Arg Met Asp Phe Ser Arg Val Thr Gly Lys
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Variable	Mean	SD	Min	Max
Age	34.5	10.2	18	65
Gender	Male	Female		
Marital status	Married	Single		
Education	High school	College		
Occupation	Manager	Worker		
Income	Low	High		
Health status	Good	Poor		
Stress level	Low	High		
Life satisfaction	Low	High		
Resilience	Low	High		
Optimism	Low	High		
Self-efficacy	Low	High		
Perceived social support	Low	High		
Perceived stress	Low	High		
Depression	Low	High		
Anxiety	Low	High		
Quality of life	Low	High		
Health-related quality of life	Low	High		
Physical health	Low	High		
Mental health	Low	High		
Overall health	Low	High		
Life expectancy	Low	High		
Healthcare utilization	Low	High		
Health insurance	Low	High		
Healthcare access	Low	High		
Healthcare quality	Low	High		
Healthcare cost	Low	High		
Healthcare satisfaction	Low	High		
Healthcare utilization	Low	High		
Healthcare access	Low	High		
Healthcare quality	Low	High		
Healthcare cost	Low	High		
Healthcare satisfaction	Low	High		

Variable	Mean	Standard Deviation	Minimum	Maximum
Age	34.5	10.2	21	55
Gender	0.5	0.5	0	1
Marital Status	0.7	0.5	0	1
Education	12.5	1.5	9	16
Income	3500	1500	1000	8000
Health	0.8	0.3	0	1
Smoking	0.2	0.4	0	1
Alcohol	0.1	0.3	0	1
Exercise	0.3	0.5	0	1
Stress	0.6	0.4	0	1
Sleep	0.7	0.3	0	1
Work	0.8	0.2	0	1
Family	0.9	0.1	0	1
Friends	0.7	0.4	0	1
Hobbies	0.5	0.5	0	1
Travel	0.4	0.5	0	1
Volunteering	0.3	0.5	0	1
Religion	0.6	0.4	0	1
Politics	0.5	0.5	0	1
Environment	0.7	0.3	0	1
Technology	0.8	0.2	0	1
Art	0.4	0.5	0	1
Music	0.6	0.4	0	1
Gardening	0.3	0.5	0	1
Cooking	0.5	0.5	0	1
Reading	0.7	0.3	0	1
Writing	0.4	0.5	0	1
Learning	0.6	0.4	0	1
Teaching	0.3	0.5	0	1
Managing	0.5	0.5	0	1
Leading	0.4	0.5	0	1
Organizing	0.6	0.4	0	1
Planning	0.5	0.5	0	1
Executing	0.7	0.3	0	1
Evaluating	0.4	0.5	0	1
Monitoring	0.6	0.4	0	1
Controlling	0.5	0.5	0	1
Improving	0.7	0.3	0	1
Maintaining	0.4	0.5	0	1
Protecting	0.6	0.4	0	1
Securing	0.5	0.5	0	1
Supporting	0.7	0.3	0	1
Assessing	0.4	0.5	0	1
Analysing	0.6	0.4	0	1
Designing	0.5	0.5	0	1
Developing	0.7	0.3	0	1
Implementing	0.4	0.5	0	1
Testing	0.6	0.4	0	1
Deploying	0.5	0.5	0	1
Operating	0.7	0.3	0	1
Managing	0.4	0.5	0	1
Administering	0.6	0.4	0	1
Supervising	0.5	0.5	0	1
Coordinating	0.7	0.3	0	1
Facilitating	0.4	0.5	0	1
Participating	0.6	0.4	0	1
Contributing	0.5	0.5	0	1
Engaging	0.7	0.3	0	1
Involving	0.4	0.5	0	1
Empowering	0.6	0.4	0	1
Mentoring	0.5	0.5	0	1
Coaching	0.7	0.3	0	1
Teaching	0.4	0.5	0	1
Learning	0.6	0.4	0	1
Researching	0.5	0.5	0	1
Writing	0.7	0.3	0	1
Editing	0.4	0.5	0	1
Proofreading	0.6	0.4	0	1
Formatting	0.5	0.5	0	1
Designing	0.7	0.3	0	1
Developing	0.4	0.5	0	1
Implementing	0.6	0.4	0	1
Testing	0.5	0.5	0	1
Deploying	0.7	0.3	0	1
Operating	0.4	0.5	0	1
Managing	0.6	0.4	0	1
Administering	0.5	0.5	0	1
Supervising	0.7	0.3	0	1
Coordinating	0.4	0.5	0	1
Facilitating	0.6	0.4	0	1
Participating	0.5	0.5	0	1
Contributing	0.7	0.3	0	1
Engaging	0.4	0.5	0	1
Involving	0.6	0.4	0	1
Empowering	0.5	0.5	0	1
Mentoring	0.7	0.3	0	1

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<212> PRT
<213> Rattus sp.

<400> 57
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35 40 45
Arg Lys Gly Gln Val Pro Ala Glu Val Val Pro Asp Pro Met Asp Met
50 55 60
Ser Leu Asp Lys Ala Glu Ala Ala Leu Val Ala Lys Glu Leu Arg Thr
65 70 75 80
Leu Leu Glu Glu Ala Val Pro Leu Ser Cys Thr Leu Pro Lys Val Thr
85 90 95
Leu Pro Asn Tyr Asp Asn Val Pro Gly Asn Leu Met Leu Ser Ala Leu
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Gly Leu Arg Leu Gly Asp Arg Val Leu Leu Asp Gly Gln Lys Thr Gly
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Thr Leu Arg Phe Cys Gly Thr Thr Glu Phe Ala Ser Gly Gln Trp Val
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Gly Val Glu Leu Asp Glu Pro Glu Gly Lys Asn Asp Gly Ser Val Gly
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Gly Val Arg Tyr Phe Ile Cys Pro Pro Lys Gln Gly Leu Phe Ala Ser
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Val Ser Lys Val Ser Lys Ala Val Asp Ala Pro Pro Ser Ser Val Thr
180 185 190

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 Thr Leu Glu Val Ala Lys Leu Ile Lys Asp Asp Phe Leu Gln Gln Asn
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 Gly Tyr Thr Pro Tyr Asp Arg Phe Cys Pro Phe Tyr Lys Thr Val Gly
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 Glu Thr Thr Ala Gln Ser Asp Asn Lys Ile Thr Trp Ser Ile Ile Arg
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 gag cac atg ggg gag att ctc tat aaa ctt tcc tcc atg aaa ttc aag 720
 Glu His Met Gly Glu Ile Leu Tyr Lys Leu Ser Ser Met Lys Phe Lys
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 Asp Pro Val Lys Asp Gly Glu Ala Lys Ile Lys Ala Asp Tyr Ala Gln
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<211> 270

<212> PRT

<213> Rattus sp.

<400> 59

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Ala	Arg	Leu	Ala	Ser	Phe	Tyr	Glu	Arg	Ala	Gly	Arg	Val	Lys	Cys	Leu
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Gly	Asn	Pro	Glu	Arg	Glu	Gly	Ser	Val	Ser	Ile	Val	Gly	Ala	Val	Ser
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Ala	Leu	Asp 115	Glu	Tyr	Tyr	Asp	Lys 120	His	Phe	Thr	Glu	Phe 125	Val	Pro	Leu
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Thr	Leu	Glu	Val	Ala 165	Lys	Leu	Ile	Lys	Asp 170	Asp	Phe	Leu	Gln	Gln 175	Asn
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Leu	Leu	Glu	Asp 260	Met	Gln	Asn	Ala	Phe 265	Arg	Ser	Leu	Glu	Asp 270		

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gag ctg ctg gag cac tac gtg gcg gcg ccg cgc cgc atg ttg ggg gcc 912
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290 295 300

cca ctg cgc cag cgc cgc gtg cgg ccg ctg cag gag ctg tgt cgc cag 960
Pro Leu Arg Gln Arg Arg Val Arg Pro Leu Gln Glu Leu Cys Arg Gln
305 310 315 320

cgc atc gtg gcc gcc gtg ggt cgc gag aac ctg gca cgc atc cct ctt 1008
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 325 330 335

aac ccg gta ctc cgt gac tac ctg agt tcc ttc ccc ttc cag atc 1053
Asn Pro Val Leu Arg Asp Tyr Leu Ser Ser Phe Pro Phe Gln Ile
340 345 350

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ggtgtggagg gtgagatgcc tcccacttct ggctggagac cttatccgc ctctcggggg 1233

gcctcccctc ctggtgctcc ctcccggtec cctgggttgt agcagcttgt gtctggggcc 1293

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ccaggggtgg gggaggggtct ctggcttcat ttttctgctg tgcagaatat tctattttat 1413

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<210> 61
<211> 351
<212> PRT
<213> Rattus sp.
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20 25 30

Ser Arg Arg Gln Pro Arg Gly Gly Lys Pro Pro Ser Gly Asp Gly Leu
35 40 45

Glu Ser Gly Pro Ser Pro Arg Pro Leu Leu His Ala Arg Gly Glu Ala
50 55 60

Gly Leu His Arg Gln Ser Gly Arg Val Pro His Thr Gly Thr Ala Tyr
65 70 75 80

Phe Ala Asp Glu Pro Thr Glu Ala Gln Ala Pro Gly Gly Phe Cys Val
85 90 95

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Project ID	Project ID	987654321
Project Manager	Project Manager	123456789
Project Status	Project Status	123456789
Project Start Date	Project Start Date	123456789
Project End Date	Project End Date	123456789
Project Budget	Project Budget	123456789
Project Location	Project Location	123456789
Project Description	Project Description	123456789
Project Objectives	Project Objectives	123456789
Project Risks	Project Risks	123456789
Project Deliverables	Project Deliverables	123456789
Project Milestones	Project Milestones	123456789
Project Stakeholders	Project Stakeholders	123456789
Project Communication	Project Communication	123456789
Project Reporting	Project Reporting	123456789
Project Documentation	Project Documentation	123456789
Project Tools	Project Tools	123456789
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Project Hardware	Project Hardware	123456789
Project Network	Project Network	123456789
Project Security	Project Security	123456789
Project Compliance	Project Compliance	123456789
Project Governance	Project Governance	123456789
Project Quality	Project Quality	123456789
Project Performance	Project Performance	123456789
Project Efficiency	Project Efficiency	123456789
Project Effectiveness	Project Effectiveness	123456789
Project Impact	Project Impact	123456789
Project Sustainability	Project Sustainability	123456789
Project Innovation	Project Innovation	123456789
Project Creativity	Project Creativity	123456789
Project Collaboration	Project Collaboration	123456789
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Project Leadership	Project Leadership	123456789
Project Management	Project Management	123456789
Project Planning	Project Planning	123456789
Project Execution	Project Execution	123456789
Project Monitoring	Project Monitoring	123456789
Project Evaluation	Project Evaluation	123456789
Project Review	Project Review	123456789
Project Feedback	Project Feedback	123456789
Project Improvement	Project Improvement	123456789
Project Success	Project Success	123456789
Project Failure	Project Failure	123456789
Project Lessons Learned	Project Lessons Learned	123456789
Project Best Practices	Project Best Practices	123456789
Project Case Studies	Project Case Studies	123456789
Project Research	Project Research	123456789
Project Analysis	Project Analysis	123456789
Project Synthesis	Project Synthesis	123456789
Project Conclusion	Project Conclusion	123456789
Project Recommendation	Project Recommendation	123456789
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Project Implementation	Project Implementation	123456789
Project Evaluation	Project Evaluation	123456789
Project Review	Project Review	12345

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Pro	Gly	Ser 115	Leu	Pro	Leu	Ser	Pro 120	Pro	Ser	Ala	Gln	Pro 125	Arg	Thr	Leu
Trp	Pro 130	Thr	Pro	Pro	Ala	Gly 135	Pro	Ser	Ser	Arg	Met 140	Val	Ala	Arg	Asn
Gln 145	Val	Ala	Ala	Asp	Asn 150	Ala	Ile	Ser	Pro	Ala 155	Ser	Glu	Pro	Arg	Arg 160
Arg	Pro	Glu	Pro	Ser 165	Ser	Ser	Ser	Ser 170	Ser	Ser	Ser	Pro	Ala	Ala 175	Pro
Ala	Arg	Pro	Arg 180	Pro	Cys	Pro	Val	Val 185	Pro	Ala	Pro	Ala	Pro 190	Gly	Asp
Thr	His	Phe 195	Arg	Thr	Phe	Arg	Ser 200	His	Ser	Asp	Tyr	Arg 205	Arg	Ile	Thr
Arg	Thr 210	Ser	Ala	Leu	Leu	Asp 215	Ala	Cys	Gly	Phe	Tyr 220	Trp	Gly	Pro	Leu
Ser 225	Val	His	Gly	Ala	His 230	Glu	Arg	Leu	Arg	Ala 235	Glu	Pro	Val	Gly	Thr 240
Phe	Leu	Val	Arg	Asp 245	Ser	Arg	Gln	Arg	Asn 250	Cys	Phe	Phe	Ala	Leu 255	Ser
Val	Lys	Met	Ala 260	Ser	Gly	Pro	Thr	Ser 265	Ile	Arg	Val	His	Phe	Gln	Ala
Gly	Arg	Phe 275	His	Leu	Asp	Gly	Ser 280	Arg	Glu	Thr	Phe	Asp 285	Cys	Leu	Phe
Glu 290	Leu	Leu	Glu	His	Tyr	Val 295	Ala	Ala	Pro	Arg	Arg 300	Met	Leu	Gly	Ala
Pro 305	Leu	Arg	Gln	Arg	Arg 310	Val	Arg	Pro	Leu	Gln 315	Glu	Leu	Cys	Arg	Gln 320
Arg	Ile	Val	Ala	Ala 325	Val	Gly	Arg	Glu	Asn 330	Leu	Ala	Arg	Ile	Pro 335	Leu
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<210> 62
<211> 1194
<212> DNA
<213> Rattus sp.

<220>
<221> CDS
<222> (130)..(765)

<400> 62
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Figure 1 consists of 12 histograms, labeled (a) through (l), arranged in a 4x3 grid. Each histogram shows the frequency of the number of non-zero elements in the vector of the first 1000 rows of the matrix A . The x-axis for all histograms is 'Number of non-zero elements' ranging from 0 to 1000. The y-axis is 'Frequency' ranging from 0 to 100. The distributions are roughly bell-shaped and centered around 500-600 non-zero elements. The histograms are labeled (a) through (l) in the top right corner of each plot.

agtgggagcg ccttattatt tcttattatt aattattatt atttttctgg aaccacgtgg 855
gagccctccc cgcctaggtc ggagggagtg ggtgtggagg gtgagatgcc tcccacttct 915
ggctggagac cttatcccgc ctctcggggg gctccccctc ctggtgctcc ctcccgtcc 975
ccctggttgt agcagcttgt gtctggggcc aggacctgaa ctccacgct acctctccat 1035
gtttacatgt tccagtatc tttgcacaaa ccaggggtgg gggaggggtct ctggcttcat 1095
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tattatgaaa gttttttttt taaaaaaaaa aaaaaaaaaa 1194

<210> 63
<211> 212
<212> PRT
<213> Rattus sp.

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1 5 10 15
Ser Glu Pro Arg Arg Arg Pro Glu Pro Ser Ser Ser Ser Ser Ser Ser
20 25 30
Ser Pro Ala Ala Pro Ala Arg Pro Arg Pro Cys Pro Val Val Pro Ala
35 40 45
Pro Ala Pro Gly Asp Thr His Phe Arg Thr Phe Arg Ser His Ser Asp
50 55 60
Tyr Arg Arg Ile Thr Arg Thr Ser Ala Leu Leu Asp Ala Cys Gly Phe
65 70 75 80
Tyr Trp Gly Pro Leu Ser Val His Gly Ala His Glu Arg Leu Arg Ala
85 90 95
Glu Pro Val Gly Thr Phe Leu Val Arg Asp Ser Arg Gln Arg Asn Cys
100 105 110
Phe Phe Ala Leu Ser Val Lys Met Ala Ser Gly Pro Thr Ser Ile Arg
115 120 125
Val His Phe Gln Ala Gly Arg Phe His Leu Asp Gly Ser Arg Glu Thr
130 135 140
Phe Asp Cys Leu Phe Glu Leu Leu Glu His Tyr Val Ala Ala Pro Arg
145 150 155 160
Arg Met Leu Gly Ala Pro Leu Arg Gln Arg Arg Val Arg Pro Leu Gln
165 170 175
Glu Leu Cys Arg Gln Arg Ile Val Ala Ala Val Gly Arg Glu Asn Leu
180 185 190
Ala Arg Ile Pro Leu Asn Pro Val Leu Arg Asp Tyr Leu Ser Ser Phe
195 200 205
Pro Phe Gln Ile

09670556-092200

210

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<212> DNA
<213> Rattus sp.

<220>
<221> CDS
<222> (52)..(336)

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Met Pro
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Ser Gln Met Glu His Ala Met Glu Thr Met Met Leu Thr Phe His Arg
5 10 15

ttt gca ggg gaa aaa aac tac ttg aca aag gag gac ctg aga gtg ctc 153
Phe Ala Gly Glu Lys Asn Tyr Leu Thr Lys Glu Asp Leu Arg Val Leu
20 25 30

atg gaa agg gag ttc cct ggg ttt ttg gaa aat caa aag gac cct ctg 201
Met Glu Arg Glu Phe Pro Gly Phe Leu Glu Asn Gln Lys Asp Pro Leu
35 40 45 50

gct gtg gac aaa ata atg aaa gac ctg gac cag tgc cga gat gga aaa 249
Ala Val Asp Lys Ile Met Lys Asp Leu Asp Gln Cys Arg Asp Gly Lys
55 60 65

gtg ggc ttc cag agc ttt cta tca cta gtg gcg ggg ctc atc att gca 297
Val Gly Phe Gln Ser Phe Leu Ser Leu Val Ala Gly Leu Ile Ile Ala
70 75 80

tgc aat gac tat ttt gta gta cac atg aag cag aag aag taggccaact 346
Cys Asn Asp Tyr Phe Val Val His Met Lys Gln Lys Lys
85 90 95

ggagccctgg taccacacacc ttgatgcgtc ctctcccatg gggtaactg aggaatctgc 406
cccaactgctt cctgtgagca gatcaggacc cttaggaaat gtgcaaataa catccaactc 466
caattcgaca agcagagaaa gaaaagttaa tccaatgaca gaggagcttt cgagttttat 526
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aaaaaaaaaa aaaa 600

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<212> PRT
<213> Rattus sp.

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Met Pro Ser Gln Met Glu His Ala Met Glu Thr Met Met Leu Thr Phe
1 5 10 15


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<210> 67
<211> 212
<212> PRT
<213> Rattus sp.

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Val Gly Lys Ser Cys Leu Leu Leu Gln Phe Thr Asp Lys Arg Phe Gln
      20           25           30
Pro Val His Asp Leu Thr Ile Gly Val Glu Phe Gly Ala Arg Met Ile
      35           40           45
Thr Ile Asp Gly Lys Gln Ile Lys Leu Gln Ile Trp Asp Thr Ala Gly
      50           55           60
Gln Glu Ser Phe Arg Ser Ile Thr Arg Ser Tyr Tyr Arg Gly Ala Ala
      65           70           75           80
Gly Ala Leu Leu Val Tyr Asp Ile Thr Arg Arg Asp Thr Phe Asn His
      85           90           95
Leu Thr Thr Trp Leu Glu Asp Ala Arg Gln His Ser Asn Ser Asn Met
      100          105          110
Val Ile Met Leu Ile Gly Asn Lys Ser Asp Leu Glu Ser Arg Arg Glu
      115          120          125
Val Lys Lys Glu Glu Gly Glu Ala Phe Ala Arg Glu His Gly Leu Ile
      130          135          140
Phe Met Glu Thr Ser Ala Lys Thr Ala Ser Asn Val Glu Glu Ala Phe
      145          150          155          160
Ile Asn Thr Ala Lys Glu Ile Tyr Glu Lys Ile Gln Glu Gly Val Phe
      165          170          175

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Asp Ile Asn Asn Glu Ala Asn Gly Ile Lys Ile Gly Pro Gln His Ala
180 185 190

Ala Thr Asn Ala Ser His Gly Gly Asn Gln Gly Gly Gln Gln Ala Gly
195 200 205

Gly Gly Cys Cys
210

<210> 68

<211> 816

<212> DNA

<213> Rattus sp.

 $\langle 220 \rangle$

<221> CDS

 $\langle 222 \rangle \quad (1) \dots (813)$

<400> 68

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1 5 10 15

gag tat caa gtg ggg cag ctg tac tot gtg gct gaa gcc agt aaa aat 96
Glu Tyr Gln Val Gly Gln Leu Tyr Ser Val Ala Glu Ala Ser Lys Asn
20 25 30

gaa act ggt ggt ggg gaa ggt gtg gag gtc ctg gtg aac gag ccc tac 144
Glu Thr Gly Gly Gly Glu Gly Val Glu Val Leu Val Asn Glu Pro Tyr
35 40 45

gag aag gat gat ggc gag aaa ggc cag tac aca cac aag atc tac cac 192
Glu Lys Asp Asp Gly Glu Lys Gly Gln Tyr Thr His Lys Ile Tyr His
50 55 60

tta	cag	agc	aaa	gtt	ccc	acg	ttt	gtt	cga	atg	ctg	gcc	cca	gaa	ggc	240
Leu	Gln	Ser	Lys	Val	Pro	Thr	Phe	Val	Arg	Met	Leu	Ala	Pro	Glu	Gly	
65					70					75					80	

gcc ctg aat ata cat gag aaa gcc tgg aat gcc tac cct tac tgc aga 288
Ala Leu Asn Ile His Glu Lys Ala Trp Asn Ala Tyr Pro Tyr Cys Arg
85 90 95

acc gtt att aca aat gag tac atg aag gaa gac ttt ctc att aaa att 336
Thr Val Ile Thr Asn Glu Tyr Met Lys Glu Asp Phe Leu Ile Lys Ile
100 105 110

gaa acc tgg cac aag cca gac ctt ggc acc cag gag aat gtg cat aaa 384
Glu Thr Trp His Lys Pro Asp Leu Gly Thr Gln Glu Asn Val His Lys
115 120 125

ctg gag cct gag gca tgg aaa cat gtg gaa gct ata tat ata gac atc 432
Leu Glu Pro Glu Ala Trp Lys His Val Glu Ala Ile Tyr Ile Asp Ile
130 135 140

gct gat cga agc caa gta ctt agc aag gat tac aag gca gag gaa gac 480
Ala Asp Arg Ser Gln Val Leu Ser Lys Asp Tyr Lys Ala Glu Glu Asp
145 150 155 160

cca gca aaa ttt aaa tct atc aaa aca gga cga gga cca ttg ggc ccg 528

Variable	Mean	SD	Min	Max
Age	34.5	10.2	21	55
Gender	0.5	0.5	0	1
Marital status	0.6	0.5	0	1
Education	12.5	1.5	9	16
Income	1500	500	500	3000
Health status	0.8	0.2	0	1
Smoking status	0.3	0.5	0	1
Alcohol consumption	0.2	0.4	0	1
Exercise frequency	0.5	0.5	0	1
Stress level	0.7	0.3	0	1
Sleep quality	0.6	0.4	0	1
Work satisfaction	0.5	0.5	0	1
Life satisfaction	0.6	0.4	0	1
Depression score	0.3	0.5	0	1
Anxiety score	0.2	0.4	0	1
Overall well-being	0.5	0.5	0	1

Pro Ala Lys Phe Lys Ser Ile Lys Thr Gly Arg Gly Pro Leu Gly Pro
165 170 175

aat tgg aag caa gaa ctt gtc aat cag aag gac tgc cca tat atg tgt 576
Asn Trp Lys Gln Glu Leu Val Asn Gln Lys Asp Cys Pro Tyr Met Cys
180 185 190

gca tac aaa ctg gtt act gtc aag ttc aag tgg tgg ggc ttg cag aac 624
Ala Tyr Lys Leu Val Thr Val Lys Phe Lys Trp Trp Gly Leu Gln Asn
195 200 205

aaa gtg gaa aac ttt ata cat aag caa gag aag cgt ctg ttt aca aac 672
Lys Val Glu Asn Phe Ile His Lys Gln Glu Lys Arg Leu Phe Thr Asn
210 215 220

ttt	cac	agg	cag	ctg	ttc	tgt	tgg	ctt	gat	aaa	tgg	gtt	gat	ctg	act	720
Phe	His	Arg	Gln	Leu	Phe	Cys	Trp	Leu	Asp	Lys	Trp	Val	Asp	Leu	Thr	
225					230					235					240	

atg gat gac att cgg agg atg gaa gaa gag acg aag aga cag ctg gat 768
Met Asp Asp Ile Arg Arg Met Glu Glu Glu Thr Lys Arg Gln Leu Asp
245 250 255

gag atg aga caa aag gac ccc gtg aaa gga atg aca gca gat gac tag 816
Glu Met Arg Gln Lys Asp Pro Val Lys Gly Met Thr Ala Asp Asp
260 265 270

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<210> 69
<211> 2263
<212> DNA
<213> Simian sp.
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catgaacttg gaagggcttg aaatgatagc agttctgacg gtcattgtgc tttttgttaa 180
attattggaa cagtttgggc tgattgaagc aggtttagaa gacagcgtgg aagatgaact 240
ggagatggcc actgtcaggc atcggcctga ggcccttgag cttctggaag ccagagcaa 300
attaccaag aaagagcttc agatccttta cagaggattt aagaacgaat gcccagtg 360
tgttgttaat gaagaaacct tcaaagagat ttactcgagc ttctttccac agggagactc 420
tacaacatat gcacattttc tgttcaatgc gtttgatacg gaccacaatg gagctgtgag 480
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gcttgatata atgaaagcaa tatacgacat gatgggtaaa tgtacatatc ctgtcctcaa 660
agaagatgca ccagacaaac acgtcgaaac attttttcag aaaatggaca aaaataaaga 720
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<211> 229

<212> PRT

<213> Simian sp.

<400> 70

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10

15

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gactctacaa catatgcaca ttttctgttc aatgcgtttg atacggacca caatggagct 480
gtgagtttcg aggatttcat caaaggctctt tccattttgc tccggggggac agtacaagaa 540
aaactcaatt gggcatttaa tctgtatgat ataaataaag atggctacat cactaaagag 600
gaaatgcttg atataatgaa agcaatatac gacatgatgg gttaaattgtac atatcctgtc 660
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tgctcaacaa ctaatccaga ttacaatatg atttagtgca tcataaaatt ccaacaattc 1920
agattatttt taatcacctc agccacaact gttaaagttgc cacattacta aagacacaca 1980
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gcaactgtct ttgcaacaat aaatcaggta tctattctgg tgtagagata ggatgttgaa 2100
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00430-964960

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<210> 72
<211> 250
<212> PRT
<213> Simian sp.
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			20					25					30		
Ile	Lys	Glu	Arg	Leu	Met	Lys	Leu	Leu	Pro	Cys	Ser	Ala	Ala	Lys	Thr
		35					40					45			
Ser	Ser	Pro	Ala	Ile	Gln	Asn	Ser	Val	Glu	Asp	Glu	Leu	Glu	Met	Ala
	50					55					60				
Thr	Val	Arg	His	Arg	Pro	Glu	Ala	Leu	Glu	Leu	Leu	Glu	Ala	Gln	Ser
65					70					75					80
Lys	Phe	Thr	Lys	Lys	Glu	Leu	Gln	Ile	Leu	Tyr	Arg	Gly	Phe	Lys	Asn
				85					90					95	
Glu	Cys	Pro	Ser	Gly	Val	Val	Asn	Glu	Glu	Thr	Phe	Lys	Glu	Ile	Tyr
			100					105					110		
Ser	Gln	Phe	Phe	Pro	Gln	Gly	Asp	Ser	Thr	Thr	Tyr	Ala	His	Phe	Leu
		115					120					125			
Phe	Asn	Ala	Phe	Asp	Thr	Asp	His	Asn	Gly	Ala	Val	Ser	Phe	Glu	Asp
	130					135					140				
Phe	Ile	Lys	Gly	Leu	Ser	Ile	Leu	Leu	Arg	Gly	Thr	Val	Gln	Glu	Lys
145					150					155					160
Leu	Asn	Trp	Ala	Phe	Asn	Leu	Tyr	Asp	Ile	Asn	Lys	Asp	Gly	Tyr	Ile
				165					170					175	
Thr	Lys	Glu	Glu	Met	Leu	Asp	Ile	Met	Lys	Ala	Ile	Tyr	Asp	Met	Met
			180					185					190		
Gly	Lys	Cys	Thr	Tyr	Pro	Val	Leu	Lys	Glu	Asp	Ala	Pro	Arg	Gln	His
		195					200					205			
Val	Glu	Thr	Phe	Phe	Gln	Lys	Met	Asp	Lys	Asn	Lys	Asp	Gly	Val	Val
	210					215					220				
Thr	Ile	Asp	Glu	Phe	Ile	Glu	Ser	Cys	Gln	Lys	Asp	Glu	Asn	Ile	Met
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Arg	Ser	Met	Gln	Leu	Phe	Glu	Asn	Val	Ile						
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<212> PRT
<213> Simian sp.

<400> 73
Ser Asn Ala Lys Ala Val Glu Thr Asp Val
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